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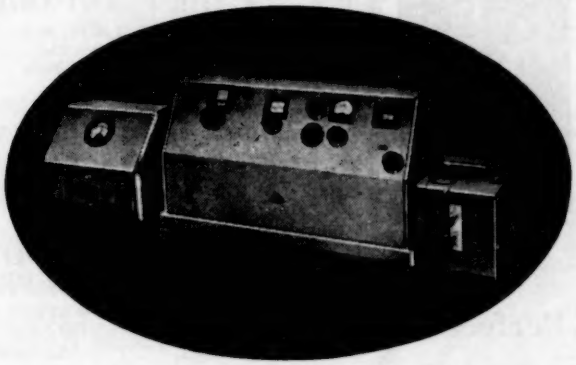

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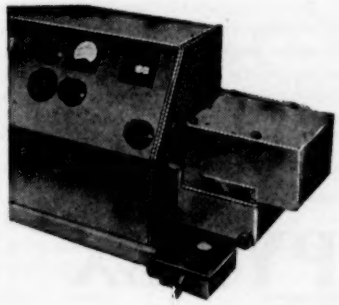

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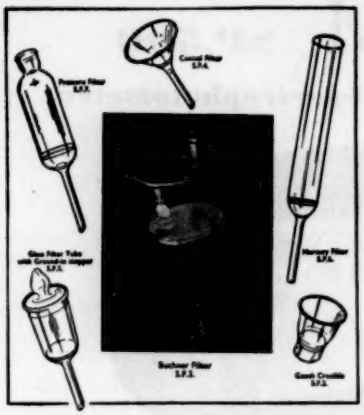

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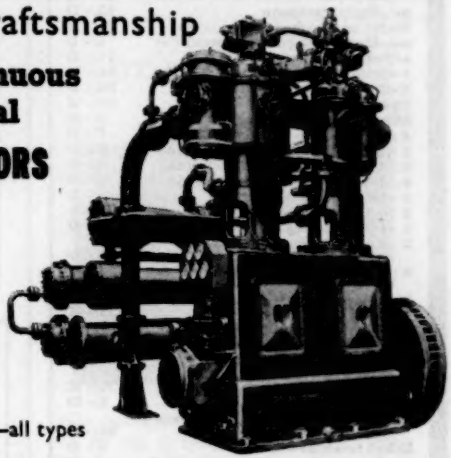

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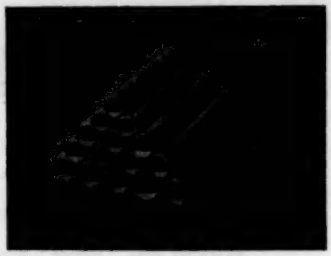

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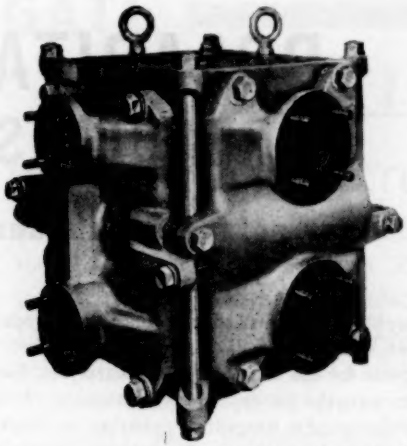

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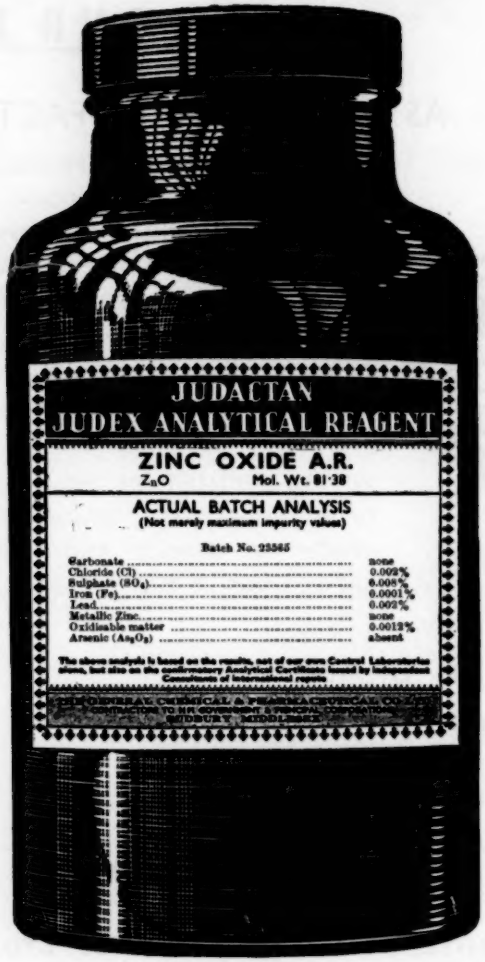
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	936	7 *	*For* the residue extracted with benzene *read* the residue boiled with water for 1 hour and extracted with benzene.
	1214		*In formula* (XII), *for* CO_2H *read* CO_2Me.
	1612	16—17	*For* converts orthoboric acid not into metaboric acid, but into boric acid *read* converts orthoboric acid into metaboric acid, but not into boric acid.
	1810	22	*For* prepared from 5-methoxytryptophan by an alternative route described below *read* derived from 5-methoxytryptophan (prepared by an alternative route described below).
	1875	1st new paragraph.	*For* M *read* μ.
		last line.	For $\left(p + \frac{a^2N^2}{V^2}\right)$ read $\left(p + \frac{aN^2}{V^2}\right)$.
	1876	28	*Delete* (curve 4).
	1919	22	For d_4^{15} read d_4^{25}.
		23	For d_4^{25} read d_4^{15}.

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ILLUSTRATIONS. 13. Illustrations accompanying the papers must be carefully drawn, preferably *twice* the size of the finished block (max. width 5 inches), *in Indian ink, on good white cartridge paper* (graph paper with faint blue lines is acceptable). Any illustration which seriously exceeds four times the size of the finished block will be returned to the author for re-drawing to a smaller scale. Lettering on the drawings, whether on the margin or in the body of the drawing, must not be in ink but must be inserted *clearly but lightly in pencil*. *Margins* of at least two inches must be left. Authors may, if they wish, submit in the first place clearly drawn pencil sketches instead of the completed diagrams, which, however, must be supplied before publication. Further information can be obtained from the Editor.

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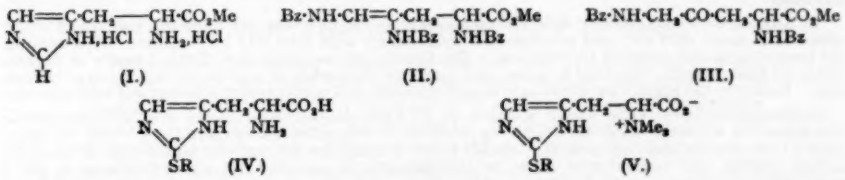
488. 2-*Mercaptoglyoxalines. Part I. The Synthesis of Ergothioneine.*

By H. Heath, Alexander Lawson, and C. Rimington.

The synthesis of ergothioneine, the only 2-mercaptoglyoxaline known to occur naturally, is described. Histidine was converted by an improved procedure (cf. Tesar and Rittenberg, *J. Biol. Chem.*, 1947, **170**, 35) into 2-mercaptohistidine, the thiol group of which was protected by conversion into the carbethoxythio-derivative. Methylation of this, to give the betaine, followed by hydrolysis and decarboxylation, yielded ergothioneine.

Ergothioneine was isolated from ergot by Tanret (*J. Pharm. Chim.*, 1909, **30**, 145; *Compt. rend.*, 1909, **149**, 222) and from blood by Newton *et al.* (*J. Biol. Chem.*, 1926, **67**, 267; 1927, **72**, 367; see also Hunter and Eagles, *ibid.*, 1925, **65**, 623; 1927, **72**, 123). It was shown to be (V; R = H) by Barger and Ewins (*J.*, 1911, **99**, 2336) and Akabori (*Ber.*, 1933, **66**, 151). Attempts to synthesize it have been made by Ashley and Harington (*J.*, 1930, 2586), Harington and Overhoff (*Biochem. J.*, 1933, **27**, 338), and Jackson and Marvel (*J. Biol. Chem.*, 1933, **103**, 191).

2-Mercaptohistidine was prepared by a modification of the procedure of Ashley and Harington (*loc. cit.*), which was based on the observation of Kossel and Edlbacher (*Z. physiol. Chem.*, 1914—15, **93**, 396) that benzoylation of histidine methyl ester dihydrochloride (I) results in fission of the glyoxaline ring to yield methyl 2 : 4 : 5-tribenzamidopent-4-enoate • (II). This on treatment with methanolic hydrogen chloride yields methyl 2 : 5-dibenzamido-4-ketopentanoate which after hydrolysis and treatment with potassium thiocyanate cyclises to give 2-mercaptohistidine (IV; R = H). Ashley and Harington obtained a 25% yield of the tribenzamido-compound (II), but by employing a two-phase benzoylation Tesar and Rittenberg (*J. Biol. Chem.*, 1947, **170**, 35) increased the yield to 60%. The method described below has given 80% yields.



(I.) (II.) (III.)

(IV.) (V.)

Attempts by previous workers to obtain ergothioneine by methylation of 2-mercaptohistidine were unsuccessful. The two difficulties to be overcome were (*a*) histidine betaine is unstable, readily losing trimethylamine to yield urocanic acid and (*b*) 2-mercaptoglyoxalines react readily with methylating agents to yield methylthioglyoxalines.

To prevent sulphur-methylation, 2-carbethoxythiohistidine was prepared by the action of ethyl chloroformate on an ethanolic suspension of 2-mercaptohistidine. Carbethoxylation of 2-mercaptohistidine in sodium hydroxide solution or aqueous pyridine did not yield the desired product as reaction occurred with the α-amino-group. Evidence for the structure of the carbethoxythio-derivative was (i) it gave a ninhydrin reaction indicating that the side-chain α-amino-group was intact, (ii) analysis proved it to be a dihydrochloride (the free mercaptoglyoxaline nucleus is not sufficiently basic to form salts), and (iii) the ultra-violet absorption spectrum failed to show the absorption maximum at *ca.* 2580 A. characteristic of the thiol group in marcaptoglyoxaline derivatives.

Methylation of the 2-carbethoxythiohistidine was first attempted with methyl sulphate and alkali according to Carter and Melville's method (*J. Biol. Chem.*, 1940, **133**, 109). The crystalline product obtained after hydrolysis of the methylated material (to remove the carbethoxy-group) was not, however, easily purified and, though apparently a betaine with a free thiol group, it did not give the deep magenta colour characteristic of ergothioneine in the Hunter diazo-test (*Biochem. J.*, 1928, **22**, 4). Methylation was achieved under neutral con-

• Geneva numbering, $CO_2H = 1$.

ditions with silver oxide and methyl iodide. The reaction mixture thus obtained, though boiled with hydrochloric acid, still contained silver, which was removed only by hydrogen sulphide. Such formation of a silver complex was to be expected as ergothioneine hydrochloride on treatment with silver nitrate forms the complex $(AgCl)_2(C_9H_{15}N_3SO_2)_2Ag_2O$ (Tanret, *loc. cit.*). The ergothioneine isolated was identical in all respects, except optical activity, with the natural material obtained from ergot. The optical activity of the 2-mercaptohistidine ($[\alpha]_D$ $-10°$) was the same as that observed by Ashley and Harington and by Tesar and Rittenberg, who proved by reconversion into L-histidine that racemization did not take place during the synthesis of 2-mercaptohistidine. Partial racemization of the ergothioneine ($[\alpha]_D$ $+47°$ instead of $+110°$) must have occurred during the formation of the betaine.

Experimental.

All m. p.s are uncorrected. Analyses are by Drs. Weiler and Strauss.

Methyl 2 : 4 : 5-Tribenzamidopent-4-enoate.—Histidine methyl ester dihydrochloride (24·1 g., 0·1 mol.) was dissolved in water (600 ml.) and cooled in an ice-salt bath. Redistilled benzoyl chloride (110 ml., 0·9 mol.) and benzene (400 ml.) were added with mechanical stirring. Sodium carbonate decahydrate (286 g., 1·0 mol.) was added in small portions during 2 hours, the cold mixture being stirred continuously for a further 5 hours. Water (350 ml.) was then added and, after filtration, the aqueous layer was extracted with benzene. The bulked benzene extracts, after being washed with water and dried (Na_2SO_4), were concentrated under reduced pressure to remove most of the benzene. Ether (2 l.) was added, and the precipitated resinous tribenzamido-compound set aside at $-10°$ overnight. The ether was decanted and the residue washed with ether. The crude product was heated in ethanol (150 ml.); it first dissolved, but rapidly crystallised when the solution was boiled; ether (300 ml.) was added and after being kept overnight at $-10°$ the crystals of methyl 2 : 4 : 5-tribenzamidopent-4-enoate (m. p. 219°; 37·9 g., 80·5%) were filtered off and washed with ether. A small quantity could be obtained from the mother-liquor by concentration under reduced pressure and addition of ether. The product thus obtained was pure enough for the next stage in the synthesis.

Methyl 2 : 5-Dibenzamido-4-ketopentanoate.—Methyl 2 : 4 : 5-tribenzamidopent-4-enoate (34 g.) was refluxed on a boiling water-bath with methanol containing 10% (w/v) of hydrogen chloride (475 ml.) for 0·5 hour. The solution was cooled and distilled under vacuum until crystals appeared. Ether (100 ml.), then iced water (400 ml.), were added. A white precipitate of methyl 2 : 5-dibenzamido-4-ketopentanoate, m. p. 158° (22·75 g., 85·7%), was formed, and on shaking complete precipitation took place. After being kept at 4° the precipitate of keto-ester was filtered off and washed with water and ether.

2 : 5-Diamino-4-ketopentanoic Acid Dihydrochloride.—The foregoing ester (65 g.) was boiled under reflux with water (300 ml.) and concentrated hydrochloric acid (350 ml.) for 7 hours. After cooling, the benzoic acid was removed by filtration. The filtrate and washings were distilled nearly to dryness under reduced pressure, dissolved in water, and extracted with ether to remove the last traces of benzoic acid. Owing to the instability of the acid dihydrochloride this concentrated solution was used directly.

2-Mercaptohistidine.—The above solution of 2 : 5-diamino-4-ketopentanoic acid dihydrochloride was heated on a boiling water-bath with the addition of potassium thiocyanate (10 g. every 0·5 hour, until a total of 40 g. had been added). After 3 hours' heating the solution was boiled with decolorizing carbon, filtered, and concentrated under reduced pressure to about 75 ml. On adjustment to pH 5 with sodium carbonate, crystallization rapidly set in, and after storage at 4° the almost colourless 2-mercaptohistidine (19·3 g., 58%) was filtered off, washed with water, and dried. It decomposed at 300° without melting. The mother-liquor darkened considerably on storage, even at 4°, and although this did not affect the crystals of 2-mercaptohistidine it was not possible to isolate any further quantity by means of mercuric chloride solution.

2-Carbethoxythiohistidine Dihydrochloride.—2-Mercaptohistidine (4·6 g.) was suspended in ethanol (100 ml.), and ethyl chloroformate (5 ml.) slowly added. This suspension was refluxed on a boiling water-bath until dissolution was effected. After cooling, dry ether (150 ml.) was added, and on storage at $-10°$ crystallization occurred. The almost colourless crystals of *2-carbethoxythiohistidine dihydrochloride* (5·8 g., 71%), m. p. 189° (decomp.), were filtered off and washed with dry ether (Found: C, 33·5; H, 4·9; N, 12·5; S, 9·6; Cl, 21·2. $C_9H_{18}O_4N_3SCl_2$ requires C, 33·5; H, 4·6; N, 12·6; S, 9·6; Cl, 21·3%). 2-Carbethoxythiohistidine dihydrochloride is very soluble in water, sparingly so in methanol and ethanol. It gives a yellow colour with sulphur dioxide in aqueous solution. In water it exhibits an ultra-violet absorption maximum at 2400 A., $\varepsilon = 9{,}400$.

Ergothioneine.—2-Carbethoxythiohistidine dihydrochloride (3·32 g., 0·01 mol.) was dissolved in water (20 ml.). Freshly prepared silver oxide (12 g.) was added as an aqueous suspension (50 ml.). Methyl iodide (1·9 ml., 0·03 mol.) was added with shaking and cooling. The mixture was then mechanically shaken. After 1 hour concentrated hydrochloric acid (50 ml.) was added and the suspension centrifuged. The precipitate was washed twice with 5N-hydrochloric acid (25 ml.). The bulked solution was boiled for 2 hours, evaporated to dryness under reduced pressure, and dissolved in water (50 ml.), and the silver removed with hydrogen sulphide. After boiling and centrifugation, the supernatant liquid was treated with saturated aqueous phosphotungstic acid until all the ergothioneine was precipitated. The precipitate of ergothioneine phosphotungstate was centrifuged off and washed with water, suspended in water, and made alkaline with saturated barium hydroxide solution. The barium phosphotungstate was centrifuged off and the supernatant solution was immediately acidified with 2N-sulphuric acid. The barium phosphotungstate was extracted twice with water, and the solution

adjusted to pH 7 with barium hydroxide solution. After centrifugation and decolorisation, the solution was concentrated under reduced pressure until crystallization of the ergothioneine occurred. Recrystallised from aqueous ethanol and dried *in vacuo* over phosphoric oxide at 105° the material had m. p. 290° and $[\alpha]_D$ +47° (c = 1, l = 2, in water) (0·8 g., 30%) (Found: C, 47·1; H, 6·7; N, 18·3; S, 14·0. Calc. for $C_9H_{15}O_2N_3S$: C, 47·1; H, 6·6; N, 18·3; S, 14·0%). The ultra-violet absorption spectrum showed a maximum at 2580 A., ε = 16,000.

The synthetic and the natural ergothioneine behaved identically in the following tests: formation of a stable dihydrate and a monohydrochloride, m. p. 250° (Found: C, 40·6; H, 6·1; N, 16·0; S, 12·0; Cl, 13·39. Calc. for $C_9H_{14}O_2N_3SCl$: C, 40·7; H, 6·1; N, 15·8; S, 12·1; Cl, 13·35%); paper chromatography (R_F 0·87 in phenol, 0·32 in collidine); Hunter diazo-, Folin–Marenzi, and Mayer's reactions. The synthetic material formed the requisite precipitates with mercuric chloride, silver oxide, potassium bismuth iodide, phosphotungstic acid, and iodine. Sulphur dioxide produced a yellow solution.

2-Mercaptourocanic Acid.—The synthetic ergothioneine (1 g.) was boiled with 40% sodium hydroxide solution (25 ml.) for 3 hours, cooled, filtered, and neutralised with hydrochloric acid. After storage at 4° the pale yellow crystals of 2-mercaptourocanic acid were collected (0·62 g.). They did not melt but decomposed at 300° (Found: C, 42·5; H, 3·9; N, 16·8; S, 18·8. Calc. for $C_6H_6O_2N_2S$: C, 42·4; H, 3·6; N, 16·5; S, 18·8%). They give the typical magenta colour with the Hunter test when the final solution is adjusted to pH 10; addition of an equal volume of 50% sodium hydroxide solution is not necessary.

We express our gratitude to Mr. P. R. E. Wallace for technical assistance throughout the synthesis, to Dr. E. Work and Mr. F. Grover for the paper chromatograms, and to Dr. G. Hunter for help in isolation in the final stages. This work was performed during the tenure by one of us (H. H.) of a British Drug Houses Research Fellowship.

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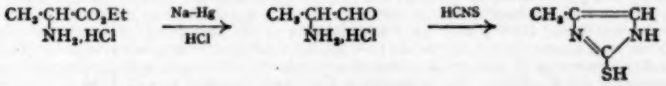
489. 2-*Mercaptoglyoxalines. Part II.** *Application of the Mannich Reaction. Protection of the Thiol Group.*

By H. Heath, Alexander Lawson, and C. Rimington.

Methods of preparing 2-mercapto-glyoxaline and -4(5)-methylglyoxaline, in larger quantities than have previously been reported, are given. The thiol group can be protected by benzylation in liquid ammonia. The Mannich reaction has been applied unsuccessfully to 2-mercaptoglyoxaline, but successfully to 2-mercapto-4(5)-methylglyoxaline to give 5(4)-dimethylaminomethyl-2-mercapto-4(5)-methylglyoxaline.

Akabori (*Ber.*, 1933, **66**, 151) prepared 2-mercapto-glyoxaline and -4(5)-methylglyoxaline from glycine and alanine respectively, by the reduction of their ethyl esters with sodium amalgam to yield the corresponding amino-aldehydes, followed by ring closure of the latter with sodium thiocyanate. By a modification of his technique a fairly large-scale preparative method for 2-mercapto-4(5)-methylglyoxaline was obtained. This compound has also been prepared from hydroxyiminoacetone by reduction with hydrogen and palladium–charcoal to aminoacetone hydrochloride and then ring closure with potassium thiocyanate, but this method is not so convenient for large quantities.



Du Vigneaud and Behrens (*J. Biol. Chem.*, 1937, **117**, 27) showed that when histidine was treated in liquid ammonia with sodium and benzyl chloride it yielded a monobenzyl derivative in which the benzyl group was attached to the glyoxaline-nitrogen atom and that the histidine could be regenerated by reductive fission with sodium in liquid ammonia. This reaction has been applied to the 2-mercaptoglyoxalines. One equivalent of sodium was rapidly absorbed by the thiol group; a second equivalent can be introduced to replace the imino-hydrogen atom,

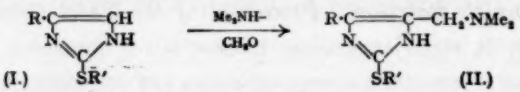
* Part I, preceding paper.

though this reaction is considerably slower. It was therefore possible to limit the reactions to the first stage. By the action of benzyl chloride on the monosodium derivatives, 2-benzylthioglyoxaline and -4(5)-methylglyoxaline were obtained in good yield.

The evidence that the compounds are *S*-benzyl and not *N*-benzyl derivatives is given by the ultra-violet absorption spectra which no longer exhibit the maxima at *ca.* 2600 A. characteristic of the free thiol group in this series, by the failure of the compounds to give the typical yellow colour with sulphur dioxide (Balaban and King, *J.*, 1927, 1858), and by their basic nature. As will be reported in a later paper the protecting groups in such alkylthioglyoxalines may be readily removed by fission with sodium in liquid ammonia. The dibenzyl derivatives of the mercaptoglyoxalines could not be obtained crystalline.

A monoacetyl-2-mercapto-4(5)-methylglyoxaline has been prepared. This substance is readily decomposed by water or alcohol with regeneration of the mercaptoglyoxaline and the change can be followed spectroscopically. In chloroform solution it is stable and unlike the 2-mercaptoglyoxalines with free thiol groups does not show an absorption maximum in the 2600-A. region. On the basis of this evidence together with its solubility in non-polar solvents, it has been assigned an acetylthio-structure. Cook, Downer, and Heilbron (*J.*, 1948, 1262) prepared two monoacetyl derivatives of 5-benzamido-2-mercaptoglyoxaline which were both stable to water and to which they tentatively assigned *N*-acetyl and *S*-acetyl structures respectively. A diacetyl product having one labile acetyl group was given the structure of a *NS*-diacetyl derivative.

The Mannich reaction has been applied to 2-mercaptoglyoxaline (I; R = R′ = H) 2-mercapto-4(5)-methylglyoxaline (I; R = Me, R′ = H), and their respective benzylthio-derivatives. Only in the case of 2-mercapto-4(5)-methylglyoxaline did the reaction proceed smoothly, yielding the expected Mannich base (II; R = Me, R′ = H). This base yields an acetate, m. p. 145°, and a picrate, m. p. 194°. It still exhibits the typical mercaptoglyoxaline properties, *e.g.*, the absorption maximum at 2600 A. and the yellow colour with sulphur dioxide. The benzylthio-derivatives yielded resins; the isolation of the picrate of 2-benzylthio-5(4)-dimethylaminomethyl-4(5)-methylglyoxaline (II; R = Me R′ = CH_2Ph) indicated that, although the reaction was complex, some of the desired product was formed.



(I.) (II.)

When the reaction was applied to 2-mercaptoglyoxaline a neutral product resulted. Addition to this of sodium carbonate caused evolution of formaldehyde, but there was no liberation of dimethylamine. The compound had typical mercaptoglyoxaline properties. The formula, $C_4H_6ON_2S$, indicated a monoformaldehyde addition compound. This was confirmed by the fact that the same substance resulted from the action of formaldehyde and hydrochloric acid alone on 2-mercaptoglyoxaline. From this evidence the structure 1-hydroxymethyl-2-mercaptoglyoxaline was assigned to this material.

Experimental.

Analyses are by Drs. Weiler and Strauss.

2-Mercapto-4(5)-methylglyoxaline.—DL-α-Alanine (50 g.) in ethanol (450 ml.) was esterified by saturation with hydrogen chloride without cooling and then refluxed for 0·5 hour; benzene (75 ml.) was added and most of the solvent removed under reduced pressure. The alanine ethyl ester was dissolved in water (700 ml.) and cooled to 0° to −5° by the addition of solid carbon dioxide. Sodium amalgam (2·5%; 2050 g.) was added in small portions with stirring, the solution being kept acid by the addition from time to time of 15% hydrochloric acid (420 ml.). The temperature was kept at 0° to −5° throughout, and stirring continued for 0·5 hour after the addition of the amalgam. The solution was filtered, boiled for 1 hour with ammonium thiocyanate (100 g.), and distilled at atmospheric pressure till crystals appeared. After storage at 4° the cream-coloured crystals of 2-mercapto-4(5)-methylglyoxaline, m. p. 246°, were separated (38 g., 59%). The absorption maximum in water was at 2580 A., ε = 14,600, and in chloroform at 2730 A., ε = 14,600. This substance when pure is colourless, not pale yellow as described in the literature.

The above substance was also prepared from hydroxyiminoacetone (10 g.) which was hydrogenated for 10 hours over palladium–charcoal (5 g. of 10%) in ethanolic 1·5N-hydrogen chloride. After removal of the solvent, water (10 ml.) and sodium thiocyanate (12 g.) were added and the solution heated on a boiling water-bath for 1 hour. On cooling the product separated. Recrystallised from water (charcoal), the crystals of 2-mercapto-4(5)-methylglyoxaline melted at 246° (4·5 g., 51%).

2-Mercaptoglyoxaline.—Glycine ethyl ester hydrochloride (100 g.) was reduced with sodium amalgam and cyclised as described above. The same quantities of amalgam, acid, and ammonium thiocyanate

were used. The resulting solution was then distilled until 500 ml. of distillate had been collected, and mercuric chloride (100 g.), dissolved in boiling water (300 ml.), was added. The mercury complex was decomposed with hydrogen sulphide to yield colourless crystals of 2-mercaptoglyoxaline, m. p. 226° (25 g., 19%). The absorption maximum in water was at 2580 A., $\varepsilon = 14{,}600$.

2-Acetylthio-4(5)-methylglyoxaline.—2-Mercapto-4(5)-methylglyoxaline (10 g.), pyridine (50 ml.), and acetic anhydride (15 ml.) were heated under reflux on a boiling water-bath for 1 hour. The solution was concentrated under reduced pressure, benzene (20 ml.) was added, and the crude acetyl compound which separated was collected and washed with benzene. The *2-acetylthio-4(5)-methylglyoxaline* recrystallised from benzene as white needles, m. p. 200°. On concentration, the mother-liquor and washings yielded a second crop, the total yield being 11·1 g. (81%). The material was soluble in ether and ethanol (Found: C, 46·3; H, 5·4; N, 17·5; S, 19·7. $C_6H_8ON_2S$ requires C, 46·2; H, 5·1; N, 18·0; S, 20·5%). The absorption maximum in chloroform was at 3250 A., $\varepsilon = 11{,}700$.

2-Benzylthio-4(5)-methylgloxaline.—2-Mercapto-4(5)-methylglyoxaline (22·8 g.) was dissolved in liquid ammonia (300 ml., approx.), and sodium (4·7 g.) was slowly added to the well-stirred mixture until the whole solution remained blue for about 20 seconds. Benzyl chloride (26 ml.) was then added dropwise and stirring continued for a further 0·5 hour. The ammonia was allowed to evaporate spontaneously. The solid residue was dissolved in 4% (w/v) hydrochloric acid (350 ml.), and the excess of benzyl chloride extracted with ether. The extracts were bulked and washed with 1% (w/v) hydrochloric acid. The solution was then made alkaline with sodium hydroxide and extracted with chloroform. The oil remaining after removal of the chloroform under reduced pressure was dissolved in ethyl acetate (40 ml.), seeded, and left overnight at −10°. The white crystals of *2-benzylthio-4(5)-methylglyoxaline*, m. p. 102° (30·6 g.), were collected. On concentration of the mother-liquor a further 4 g. were obtained (total, 85·6%). The compound is soluble in methanol, ethanol, acetic acid, pyridine, acetone, benzene, chloroform, or carbon tetrachloride, sparingly so in ether and ethyl acetate, insoluble in water and light petroleum (Found: C, 64·3; H, 6·4; N, 13·7; S, 14·8. $C_{11}H_{12}N_2S$ requires C, 64·7; H, 5·9; N, 13·7; S, 15·7%).

2-Benzylthioglyoxaline.—2-Mercaptoglyoxaline (20 g.) was converted into *2-benzylthioglyoxaline* by the procedure described for 2-benzylthio-4(5)-methylglyoxaline. The white solid residue left on evaporation of the ammonia was dissolved in 4% (w/v) hydrochloric acid (350 ml.) and extracted with ether. The aqueous layer was made alkaline with sodium hydroxide and after storage at 4° the 2-benzylthioglyoxaline was filtered off and washed with water. The dried material recrystallised from boiling benzene (1 l.) (charcoal) as very light fine white needles, m. p. 153° (34 g., 90%), soluble in ethanol, sparingly so in benzene, chloroform, or ether (Found: C, 63·5; H, 5·7; N, 14·2; S, 16·6. $C_{10}H_{10}N_2S$ requires C, 63·1; H, 5·3; N, 14·7; S, 16·8%).

5(4)-Dimethylaminomethyl-2-mercapto-4(5)-methylglyoxaline Acetate.—33% (w/v) Aqueous dimethylamine (15 ml.) was cooled to 0° and acetic acid (15 ml.) was added so that the temperature did not rise above 5°, followed by 40% aqueous formaldehyde, the temperature still being kept below 5°. To this solution, 2-mercapto-4(5)-methylglyoxaline (11·4 g.) was added with mechanical shaking at room temperature until dissolution was effected. After being kept the solution was distilled under reduced pressure to a thick syrup. The *5(4)-dimethylaminomethyl-2-mercapto-4(5)-methylglyoxaline acetate* crystallised from ethanol (120 ml.). On cooling to −10° the short white needles, m. p. 148° (15 g.), were filtered off, washed with ethanol, and dried. On concentration of the mother-liquor and washings, a second crop of crystals was obtained (total, 18·9 g., 81·8%). The substance was very soluble in water and sparingly so in ethanol (Found: C, 46·9; H, 7·5; N, 17·8; S, 13·7. $C_9H_{17}O_2N_3S$ requires C, 46·7; H, 7·4; N, 18·2; S, 13·8%).

5(4)-Dimethylaminomethyl-2-mercapto-4(5)-methylglyoxaline.—The free *base* was precipitated by treating a strong aqueous solution of the acetate with an equivalent amount of sodium carbonate solution. It is not necessary to isolate the acetate, as the thick syrup obtained in the previous preparation readily crystallised when made faintly alkaline with ammonia solution. After storage at 4° the crystals were filtered off, washed with water, and dried. The substance did not melt but decomposed at 280° (Found: C, 49·4; H, 7·7; N, 24·7; S, 18·2. $C_7H_{13}N_3S$ requires C, 49·1; H, 7·7; N, 24·5; S, 18·7%). The mother-liquors were made faintly acid with acetic acid, concentrated to dryness under reduced pressure, and dissolved in the minimum amount of water. On neutralisation with aqueous ammonia and storage at 4° a further crop of crystals (3·4 g.) was obtained. The total yield was 14·5 g. (84·7%). The base is soluble in hot ethanol, sparingly soluble in water or cold ethanol, and its absorption maximum in ethanol is at 2660 A., $\varepsilon = 15{,}000$.

1-Hydroxymethyl-2-mercaptoglyoxaline.—(Method I) A solution of dimethylamine, acetic acid, and formaldehyde prepared as above was added dropwise, with mechanical stirring, to 2-mercaptoglyoxaline (10 g.) in water (50 ml.). After storage at 4°, crystallisation commenced. The *1-hydroxymethyl-2-mercaptoglyoxaline*, m. p. 161° (9·2 g., 71%), was recrystallised from ethanol (Found: C, 37·3; H, 4·7; N, 20·8; S, 24·1. $C_4H_6ON_2S$ requires C, 36·9; H, 4·6; N, 21·5; S, 24·6%).

(Method II) 2-Mercaptoglyoxaline (5 g.) was dissolved in concentrated hydrochloric acid (30 ml.), and 40% formaldehyde solution (3·8 ml.) was slowly added. The clear solution, after storage, was distilled to dryness under reduced pressure. The solid residue was dissolved in water (5 ml.) and neutralised with sodium carbonate. Crystallisation of the 1-hydroxymethyl-2-mercaptoglyoxaline, m. p. 161° (3·9 g., 60%), rapidly occurred. The absorption maximum in water was at 2550 A., $\varepsilon = 15{,}000$.

Mannich Reaction with 2-Benzylthio-4(5)-methylglyoxaline.—A solution of dimethylamine, acetic acid, and formaldehyde was prepared as in the preparation of 5(4)-dimethylaminomethyl-2-mercapto-4(5)-methylglyoxaline acetate. This was added to 2-benzylthio-4(5)-methylglyoxaline (20·4 g.), dissolution being effected by shaking. After storage at room temperature, water (40 ml.) was added and the solution neutralised with sodium hydroxide. The precipitated gum was extracted with ether, and the extracts

were washed with water and dried (Na_2SO_4). Removal of the ether under reduced pressure left a clear, pale yellow, viscous residue which was soluble in ethanol, acetone, chloroform, ethyl acetate, benzene, or ether, and could not be obtained crystalline. Treatment with excess of ethanolic picric acid gave crystals of *2-benzylthio-5(4)-dimethylaminomethyl-4(5)-methylglyoxaline dipicrate*; recrystallised (charcoal) from boiling water (3 l.), this had m. p. 204° (Found: C, 43·4; H, 3·5. $C_{28}H_{28}O_{14}N_8S$ requires C, 43·4; H, 3·5%). The twice recrystallised picrate (3·6 g.) was shaken with N-sodium hydroxide (50 ml.) and extracted with ether. The colourless, glassy residue left on evaporation of the ether was dissolved in light petroleum (b. p. 40—60°) (75 ml.) and cooled to −10°. After 4 weeks, white crystals of *2-benzylthio-5(4)-dimethylaminomethyl-4(5)-methylglyoxaline*, m. p. 91° (0·8 g.), separated (Found: C, 64·2; H, 7·0; N, 16·2; S, 12·3. $C_{14}H_{19}N_3S$ requires C, 64·3; H, 7·3; N, 16·1; S, 12·3%).

We are grateful to Mr. R. A. F. Bullerwell of the Royal Free Hospital School of Medicine for informing us of the use of solid carbon dioxide in the Akabori reduction procedure. This work was performed during the tenure by one of us (H. H.) of a British Drug Houses Research Fellowship and forms part of a thesis for the Degree of Ph.D. in the University of London.

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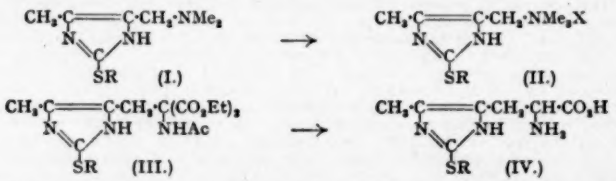
490. *2-Mercaptoglyoxalines. Part III.* Synthesis of 2-Mercapto-4(5)-methylhistidine and 4-Methylergothioneine.*

By H. Heath, Alexander Lawson, and C. Rimington.

5(4)-Dimethylaminomethyl-2-mercapto-4(5)-methylglyoxaline, after methylation with methyl sulphate, can be condensed with ethyl sodioacetamidomalonate to yield, after hydrolysis and decarboxylation 4(5)-methyl-2-methylthiohistidine. This can then be converted into 2-metcapto-4(5)-methylhistidine by treatment with sodium in liquid ammonia, and into 4-methylergothioneine by the method described in Part I.

The projected method for the synthesis of 2-mercapto-4(5)-methylhistidine was to methylate 5(4)-dimethylaminomethyl-2-mercapto-4(5)-methylglyoxaline (I; R = H), to the quaternary ammonium salt (II; R = H) and condense the latter with ethyl sodioacetamidomalonate, in accordance with the procedure used by Rydon (*J.*, 1948, 705) for the methyltryptophans. By hydrolysis and decarboxylation of this condensation product (III; R = H), 2-mercapto-4(5)-methylhistidine (IV; R = H) should result.



This synthesis was at first unsuccessful because it was not found possible to form the quaternary ammonium salt without methylating the thiol group: the dipicrate of 5(4)-dimethylaminomethyl-4(5)-methyl-2-methylthioglyoxaline (II; R = Me) was isolated. It was therefore thought advisable to protect the sulphur atom by either a methyl or preferably (on account of the ease of removal at a later stage) a benzyl group. As reported in Part II the Mannich condensation proceeds more easily with the free mercapto-compound than with the *S*-benzyl derivative. Benzylation of the Mannich base (I; R = H), however, brought about decomposition, probably owing to the instability of the dimethylaminomethyl group under alkaline conditions (cf. Mannich and Dannehl, *Arch. Pharm.*, 1938, **276**, 206).

The alternative procedure of starting with the benzylated mercaptoglyoxaline for the Mannich reaction also yielded a resinous material, which, after methylation to the quaternary ammonium compound (II; R = CH_2Ph), was condensed with ethyl sodioacetamidomalonate.

* Part II, preceding paper.

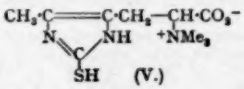
This yielded a fraction which analysis showed to be $C_{31}H_{43}O_{19}N_4S$, corresponding to the introduction of two ethyl acetamidomethylmalonate groups into the molecule.

Protection of the sulphur atom by benzylation having thus proved impracticable, the Mannich base (I; R = H) was converted into the quaternary methyl derivative with simultaneous methylation of the thiol group. The malonic ester condensation product (III; R = Me) could not be crystallised; it was hydrolysed and decarboxylated to yield 4(5)-methyl-2-methylthiohistidine (IV; R = Me). From this the pure amino-acid hydrochloride was obtained after esterification and subsequent hydrolysis.

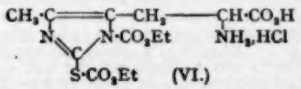
Attempts to remove the methyl group from the sulphur of the amino-acid methyl ester with sodium in liquid ammonia did not yield the expected product. The reaction was obviously not a simple demethylation; the end-point was not sharp and much more than the theoretical amount of sodium was taken up before the solution remained blue. A crystalline basic product isolated from the reaction gave the sulphur dioxide test and the ultra-violet absorption maximum characteristic of the mercaptoglyoxaline ring. The ninhydrin reaction was, however, negative, even after hydrolysis. It is possible that, in addition to the removal of the protecting group from the sulphur atom, an amide group formed from the ester by the action of the liquid ammonia was reduced to the corresponding amino-alcohol.

It has been shown by du Vigneaud and Behrens (*J. Biol. Chem.*, 1937, **117**, 27) that histidine can easily be obtained by the reductive debenzylation of N^{α}-benzylhistidine. When 4(5)-methyl-2-methylthiohistidine (IV; R = Me) was used in place of its ester, reductive fission smoothly yielded 2-mercapto-4(5)-methylhistidine (IV; R = H).

Attempts to prepare 4-methylergothioneine (V) from 2-mercapto-4(5)-methylhistidine (IV; R = H) by the method described in Part I of this series for the synthesis of ergothioneine showed again that the methyl group in the 4(5)-position has a marked influence on the behaviour of the glyoxaline ring. The preparation of the carbethoxythio-derivative gave a deliquescent material of unexpectedly low melting point, the analysis for which indicated a dicarbethoxy-derivative (VI). Methylation and decarboxylation of this compound yielded 4-methylergothioneine (V).



(V.)



(VI.)

4-Methylergothioneine responds to all the usual colour tests for ergothioneine except the Hunter diazo-reaction (*Biochem. J.*, 1928, **22**, 4). This was expected from the work of Lawson, Morley, and Woolf (*Nature*, 1951, **167**, 82) since the 4(5)-position is blocked. However, when the pH of the coupling solution in the Hunter test was lowered to about 7 by using 2% sodium acetate instead of the usual buffer, a very pale yellow primary colour resulted. On addition of the sodium hydroxide, trimethylamine was liberated and an indigo colour (absorption maximum at 5900 A.) developed. The sensitivity of this reaction is about one-tenth of the Hunter ergothioneine test.

Experimental.

Analyses are by Drs. Weiler and Strauss.

4(5)-Methyl-2-methylthiohistidine Methyl Ester Dihydrochloride.—This condensation must be carried out under strictly anhydrous conditions. 5(4)-Dimethylaminomethyl-2-mercapto-4(5)-methylglyoxaline (17·7 g.) and ethyl acetamidomalonate (25·4 g.) were suspended in ethanol (dried over magnesium–iodine; 150 ml.). A solution of sodium (5·4 g.) in ethanol (150 ml.) was added and to the resulting solution methyl sulphate (28 ml.) was added, the temperature being allowed to rise as the reaction proceeded. After the solution had been kept overnight at room temperature, water (200 ml.) was added and the solution was distilled under reduced pressure to a low volume. 25% (w/v) Sulphuric acid (250 ml.) was added and the solution boiled for 5 hours, cooled, and diluted to 500 ml. The solution was brought to pH 4 with sodium hydroxide and treated with a hot solution of mercuric chloride (100 g.) in water (500 ml.). After adjustment of the pH to 7·4 with sodium carbonate and storage overnight, the precipitate of the mercury complex of 4(5)-methyl-2-methylthiohistidine was centrifuged off and washed with water until free from sulphate. The mercury derivative was decomposed with hydrogen sulphide, and the mercury-free filtrate evaporated to dryness under reduced pressure. The dry residue was then refluxed with methanol (400 ml.), saturated with hydrogen chloride, for 1 hour whereupon crystals began to separate. After being kept at −10°, the colourless crystals of *4(5)-methyl-2-methylthiohistidine methyl ester dihydrochloride*, m. p. 224° (16·8 g., 65%) (Found: C, 36·1; H, 5·6; N, 14·2; S, 10·0. $C_9H_{17}O_2N_3SCl_2$ requires C, 35·8; H, 5·7; N, 13·9; S, 10·6%), were collected. They recrystallised from methanol in long, thin rectangular prisms. The absorption maximum was at 2550 A., ε = 7800. The compound is very soluble in water, sparingly so in methanol, and insoluble in ether.

2-Mercapto-4(5)-methylhistidine.—4(5)-Methyl-2-methylthiohistidine methyl ester dihydrochloride (10 g.) was refluxed for 1 hour with 10% hydrochloric acid (100 ml.) and then distilled to dryness under reduced pressure. The dried white crystalline residue was dissolved in liquid ammonia (400 ml.), and sodium added in small pieces until a permanent blue colour developed. Ammonium chloride (2 g.) was then added and the ammonia allowed to evaporate spontaneously. The white solid residue was dissolved in dilute sodium hydroxide solution (15 ml.). On adjustment to pH 4 with acetic acid the *2-mercapto-4(5)-methylhistidine* crystallised (3·8 g., 57%) (Found: C, 42·3; H, 5·6; N, 20·4; S, 15·7. $C_7H_{11}O_2N_3S$ requires C, 41·8; H, 5·5; N, 20·9; S, 16·0%), soluble in hot water, sparingly so in cold water, and insoluble in ethanol. The absorption maximum in water was at 2600 A., $\varepsilon = 15{,}300$. The compound crystallises from water in thin microscopic prisms which do not melt, but decompose above 300°. It gives positve reactions with ninhydrin, sulphur dioxide, mercuric chloride, and the Folin–Marenzi reagent. The Hunter diazo-test is negative.

Condensation of Mannich Base of 2-Benzylthio-4(5)-methylglyoxaline with Sodioacetamidomalonic Ester.—The viscous solution of the Mannich base prepared from 2-benzylthio-4(5)-methylglyoxaline (20·4 g.) (cf. Part II) was dissolved in ethanol (100 ml.). Ethyl sodioacetamidomalonate, prepared from ethyl acetamidomalonate (21·7 g.) by addition of ethanol (200 ml.) containing sodium (2·3 g.), was mixed with the Mannich base, and methyl sulphate (19 ml.) added. Trimethylamine was evolved and, after storage and removal of the solvent under reduced pressure, the solution was made alkaline with sodium carbonate and extracted with ether, which on evaporation left a pale yellow non-crystalline residue. This crystallised after 2 months from amyl acetate or carbon tetrachloride. The analytical figures, though not entirely satisfactory, were obtained on using material once recrystallised from amyl acetate and twice from carbon tetrachloride without change of m. p. (115°) (Found: C, 56·2; H, 6·4; N, 9·0; S, 5·3. $C_{31}H_{42}O_{10}N_4S$ requires C, 56·2; H, 6·4; N, 8·5; S, 4·8%). This *compound* is therefore a condensation product between the Mannich di-base of 2-benzylthio-4(5)-methylglyoxaline and two molecules of ethyl acetamidomalonate.

5(4)-Dimethylaminomethyl-4(5)-methyl-2-methylthioglyoxaline Dipicrate.—5(4)-Dimethylaminomethyl-2-mercapto-4(5)-methylglyoxaline (5 g.) was dissolved in ethanol (100 ml.), and methyl iodide (4·2 g.) in ethanol (10 ml.) added. After being kept at room temperature, the alcohol was removed under reduced pressure and excess of hot aqueous picric acid solution added. On cooling, short yellow needles, m. p. 201°, of *5(4)-dimethylaminomethyl-4(5)-methyl-2-methylthioglyoxaline dipicrate* separated (Found: C, 37·5; H, 3·2; N, 19·7; S, 4·7. $C_{22}H_{21}O_{14}N_8S$ requires C, 37·3; H, 3·3; N, 19·6; S, 5·0%). The formation of a dipicrate can best be accounted for on the basis of the thiol group having been methylated, thus rendering the glyoxaline ring basic.

1-Carbethoxy-2-carbethoxythio-4(5)-methylhistidine Hydrochloride.—2-Mercapto-4(5)-methylhistidine (3·8 g.), suspended in ethanol (100 ml.), was refluxed with ethyl chloroformate (4 ml.) till dissolution was effected. After cooling and addition of dry ether (150 ml.), the oil which separated crystallised rapidly when scratched. Additional material was obtained from the mother-liquor (total yield, 4·76 g., 60%). The *hydrochloride* was deliquescent and had m. p. 60° (Found: C, 36·2; H, 5·5; S, 7·5; Cl, 8·3. $C_{13}H_{20}O_6N_3SCl,2H_2O$ requires C, 37·3; H, 5·8; S, 7·7; Cl, 8·5%).

4-Methylergothioneine.—The dicarbethoxy-derivative obtained as above was treated as described in the preparation of ergothioneine (Part I). The *4-methylergothioneine* crystallised from aqueous alcohol and had m. p. 265° (decomp.) (Found: C, 48·7; H, 7·1; N, 17·2; S, 13·5. $C_{10}H_{17}O_2N_3S$ requires C, 49·4; H, 7·1; N, 17·3; S, 13·2%). This compound, like ergothioneine, was soluble in water, sparingly so in ethanol. The tests with sulphur dioxide, gold chloride, mercuric chloride, iodobismuthous acid, and the Folin–Marenzi reagent were positive. The absorption maximum in water was at 2600 A., $\varepsilon = 15{,}600$.

This work was performed during the tenure by one of us (H. H.) of a British Drug Houses Research Fellowship and forms part of a thesis for the degree of Ph.D. in the University of London. We thank Mr. P. R. E. Wallace for technical assistance.

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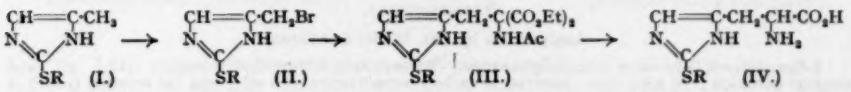
491. 2-*Mercaptoglyoxalines. Part IV.* Bromination with N-Bromosuccinimide. The Reimer–Tiemann Reaction.*

By H. Heath, Alexander Lawson, and C. Rimington.

The action of *N*-bromosuccinimide on 2-mercapto-4(5)-methylglyoxaline has been investigated under varying conditions of temperature, solvent, and sulphur protection. From 2-mercapto-4(5)-methylglyoxaline (I; R = H) and its acetylthio-derivative (I; R = Ac), di-[5(4)-bromo-4(5)-methyl-2-glyoxalinyl] disulphide is formed. From the benzylthio-derivative (I; R = CH_2Ph), 2-benzylthio-5(4)-bromo-4(5)-methylglyoxaline results. By the Reimer–Tiemann reaction with 2-mercapto-4(5)-methylglyoxaline (I; R = H), the unstable 5(4)-formyl-2-mercapto-4(5)-methylglyoxaline is formed; this is isolated as the semicarbazone.

The object of the research was to synthesise 4(5)-bromomethyl-2-mercaptoglyoxaline (II; R = H) which could then be condensed with ethyl sodioacetamidomalonate, hydrolysed, and decarboxylated to yield 2-mercaptohistidine (IV; R = H).



Unsuccessful attempts have been made by Jackson and Marvel (*J. Biol. Chem.*, 1933, **103**, 191) to synthesise 4(5)-chloromethyl-2-mercaptoglyoxaline by the action of thionyl chloride or concentrated hydrochloric acid on 4(5)-hydroxymethyl-2-mercaptoglyoxaline. In all cases a polymeric product was obtained.

The reaction between *N*-bromosuccinimide and 2-mercapto-4(5)-methylglyoxaline (I; R = H) was unsuccessful, possibly owing to the insolubility of both reactants in carbon tetrachloride. 2-Benzylthio-4(5)-methylglyoxaline reacted with *N*-bromosuccinimide and benzoyl peroxide at 0° to −5° to yield a compound, $C_{11}H_{11}N_2SBr$, m. p. 154°, which although a monobromo-derivative, was not the required 2-benzylthio-4(5)-bromomethylglyoxaline, as the halogen was very firmly attached to the molecule, not being removed by aqueous or alcoholic potassium hydroxide. With dilute nitric acid, however, bromine was readily removed with the formation of benzyl 4(5)-methyl-5(4)-nitro-2-glyoxalinyl sulphoxide, m. p. 226°.

The bromo-derivative, m. p. 154°, was assigned the structure 2-benzylthio-5(4)-bromo-4(5)-methylglyoxaline. The results of a *C*-methyl determination, together with the stability shown by the substance towards alkali, would eliminate a bromomethyl or bromomethylene structure, and the reactivity with nitric acid was hardly to be expected had the halogenation occurred in the benzene ring. It therefore seems probable that by the action of nitric acid the bromine was removed from the 5(4)-position in the glyoxaline ring and replaced by a nitro-group. Although no halogen-substituted mercaptoglyoxalines have previously been described, Wohl and Markwald (*Ber.*, 1889, **22**, 1353) have shown that by the action of nitric acid on 2-methylthioglyoxaline the 4(5)-nitro-derivative is formed.

The action of two equivalents of *N*-bromosuccinimide on 2-mercapto-4(5)-methylglyoxaline in aqueous solution yielded an amorphous pale yellow insoluble base, $C_8H_8N_4S_2Br_2$, in 91% yield. The failure of this compound to show an absorption maximum in the 2600-A. region and failure to detect a thiol group in the amperometric titration (Kolthoff and Harris, *Ind. Eng. Chem.*, 1945, **18**, 161), together with its basic nature, showed that the thiol group had been modified. The facts that the compound gave the typical sulphur dioxide reaction and could be smoothly reduced by this reagent to 5(4)-bromo-2-mercapto-4(5)-methylglyoxaline, $C_4H_5N_2SBr$, showed conclusively that the *N*-bromosuccinimide besides brominating the nucleus had also oxidised the thiol group to the disulphide. It is difficult to limit the oxidation of 2-mercaptoglyoxalines to the formation of the disulphides (Balaban and King, *J.*, 1927, 1858), since unstable sulphinic acids, which liberate sulphur dioxide to give the corresponding glyoxaline, are usually formed. The preparation of the disulphide from 2-mercapto-4 : 5-diphenylglyoxaline has, however, been

* Part III, preceding paper.

reported by Anschutz and Schwickerath (*Annalen*, 1895, **284**, 99). There is no record in the literature of the isolation of mercaptoglyoxalines by reduction of their disulphides.

5(4)-Bromo-2-mercapto-4(5)-methylglyoxaline, in contrast to the disulphide, could be crystallised from hot water and showed the usual mercaptoglyoxaline behaviour in its ultra-violet absorption spectrum and in amperometric titration. On oxidation with ferric chloride (Pyman, *J.*, 1911, **99**, 668), 4(5)-bromo-5(4)-methylglyoxaline, m. p. 152°, was formed. This had previously been prepared by Pyman and Timmis (*J.*, 1923, **123**, 494) by the bromination of 4(5)-methylglyoxaline. The bromine in this bromoglyoxaline was quite stable to alkaline hydrolysis in contrast to that in the disulphide, from which the corresponding hydroxy-compound was isolated on treatment with 2% sodium hydroxide.

Attempted bromination, by *N*-bromosuccinimide in benzene solution, of 2-acetylthio-4(5)-methylglyoxaline resulted in the cleavage of the somewhat labile acetyl group with formation of di-[5(4)-bromo-4(5)-methyl-2-glyoxalinyl] disulphide. The Reimer–Tiemann reaction has been successfully applied to 2-mercapto-4(5)-methylglyoxaline, 5(4)-formyl-2-mercapto-4(5)-methylglyoxaline being isolated as the semicarbazone, m. p. 294°. The corresponding free aldehyde could not be isolated.

No condensation product could be isolated after reaction (even at −50°) between ω-bromophthalimidoacetone and ethyl sodioacetamidomalonate (cf. Harington and Overhoff, *Biochem. J.*, 1933, **27**, 338).

Experimental.

Analyses are by Drs. Weiler and Strauss.

2-Benzylthio-5(4)-bromo-4(5)-methylglyoxaline.—2-Benzylthio-4(5)-methylglyoxaline (17·1 g.) and benzoyl peroxide (170 mg.) were dissolved in carbon tetrachloride (350 ml.), and the solution cooled in an ice–salt bath to 0° to −5°. Finely powdered *N*-bromosuccinimide (14·9 g.) was slowly added with mechanical stirring during 2 hours and the solution stirred for another hour. The pale yellow powder which was deposited was filtered off and washed with carbon tetrachloride and with cold water. Sodium carbonate solution was added to pH 7 and the insoluble *2-benzylthio-5(4)-bromo-4(5)-methylglyoxaline* (22·8 g., 96%) was collected. Recrystallised from benzene (300 ml.) (charcoal), it formed colourless crystals, m. p. 154° (Found: C, 46·4; H, 3·8; N, 9·5; S, 10·9; Br, 28·0; CMe, 3·4. $C_{11}H_{11}N_2SBr$ requires C, 46·5; H, 3·9; N, 9·9; S, 11·3; Br, 28·2; 1-CMe, 5·3%).

Benzyl 4(5)-Methyl-5(4)-nitro-2-glyoxalinyl Sulphoxide.—2-Benzylthio-5(4)-bromo-4(5)-methylglyoxaline (1·4 g.) was boiled with 3% nitric acid (30 ml.) until dissolution was effected and nitrous fumes were no longer evolved. After filtration of the hot solution, crystals of the *sulphoxide* were deposited on cooling and were recrystallised from water (charcoal). This compound is strongly adsorbed on charcoal and the minimum amount should be used. Recrystallised from ethanol it had m. p. 226° (1·0 g., 77%) (Found: C, 49·4; H, 4·3; N, 15·0. $C_{11}H_{11}O_3N_3S$ requires C, 49·8; H, 4·2; N, 15·8%). The Liebermann nitroso- and the brucine nitrate test were negative.

Di-[5(4)-bromo-4(5)-methyl-2-glyoxalinyl] Disulphide.—*N*-Bromosuccinimide (20·3 g.) in water (700 ml.) was slowly added to 2-mercapto-4(5)-methylglyoxaline (6·5 g.) in water (100 ml.) with mechanical stirring. After 1 hour sodium acetate was added to adjust the solution to pH 7 and the pale yellow precipitate of *di-[5(4)-bromo-4(5)-methyl-2-glyoxalinyl] disulphide* (10·0 g., 91%) was collected. This substance, which does not melt below 300°, was insoluble in all the usual organic solvents and was partly purified by dissolution in dilute hydrochloric acid followed by reprecipitation on adjustment of the pH with sodium acetate (Found: C, 25·6; H, 2·3; N, 14·9; S, 16·9; Br, 40·4. $C_8H_8N_4S_2Br_2$ requires C, 25·0; H, 2·1; N, 14·6; S, 16·7; Br, 41·6%); the chief remaining impurity was di-[4(5)-methyl-2-glyoxalinyl] disulphide, which behaved similarly. The product does not exhibit an absorption maximum between 2100 and 3600 A.

Di-[5(4)-hydroxy-4(5)-methyl-2-glyoxalinyl] Disulphide.—Di-[5(4)-bromo-4(5)-methyl-2-glyoxalinyl] disulphide (2 g.) was refluxed for 30 minutes with 1% aqueous sodium hydroxide (30 ml.), cooled, and filtered. On neutralisation of the filtrate, *di-[5(4)-hydroxy-4(5)-methyl-2-glyoxalinyl] disulphide* (1·2 g., 89%), m. p. 252° (decomp.), was precipitated (Found: C, 37·6; H, 4·3; N, 21·0; S, 12·8. $C_8H_{10}O_2N_4S_2$ requires C, 37·2; H, 3·9; N, 21·7; S, 12·4%). This is insoluble in all the usual organic solvents and does not exhibit an absorption maximum between 2100 and 3600 A. Addition of excess of aqueous picric acid to a solution in dilute hydrochloric acid gave a crystalline picrate which, after recrystallisation from aqueous ethanol, had m. p. 208° (decomp.).

5(4)-Bromo-2-mercapto-4(5)-methylglyoxaline.—Di-[5(4)-bromo-4(5)-methyl-2-glyoxalinyl] disulphide (0·5 g.) was dissolved in 10% hydrochloric acid (15 ml.), and sulphur dioxide was passed through the solution for 1 hour. After storage, orange crystals were collected which readily gave off sulphur dioxide, to leave pale yellow crystals of *4(5)-bromo-2-mercapto-4(5)-methylglyoxaline* (0·21 g., 42%), m. p. 246° (Found: C, 24·9; H, 2·9; N, 15·1; S, 16·7; Br, 40·7. $C_4H_5N_2SBr$ requires C, 24·9; H, 2·6; N, 14·5; S, 16·6; Br, 41·4%). This is sparingly soluble in water and dilute acids, readily so in dilute sodium hydroxide and ethanol, and does not form a picrate. It exhibits an absorption maximum in ethanol at 2700 A., $\varepsilon = 16{,}200$.

Action of N-Bromosuccinimide on 2-Acetylthio-4(5)-methylglyoxaline.—*N*-Bromosuccinimide (2·3 g.) in benzene (200 ml.) was added to 2-acetylthio-4(5)-methylglyoxaline (1·0 g.) and benzoyl peroxide (10 mg.)

in benzene (100 ml.). After storage, the pale yellow precipitate was filtered off and washed with benzene. Dissolving it in water and adjusting the pH to 7 with sodium acetate gave di-[5(4)-bromo-4(5)-methyl-2-glyoxalinyl] disulphide, identical with the material obtained from *N*-bromosuccinimide and 2-mercapto-4(5)-methylglyoxaline.

Reimer–Tiemann Reaction with 2-Mercapto-4(5)-methylglyoxaline.—2-Mercapto-4(5)-methylglyoxaline (5 g.) in 2N-sodium hydroxide (100 ml.) was refluxed for 1 hour with chloroform (5 ml.). The dark red solution was acidified with hydrochloric acid, boiled, and filtered and the green filtrate decolorised with charcoal to give a pale yellow solution, which yielded the oxime [m. p. 234° (decomp.)], the 2 : 4-dinitrophenylhydrazone (m. p. 198°), and the *semicarbazone* [m. p. 294° (decomp.)] of 5(4)-formyl-4(5)-methylglyoxaline, which were all very sparingly soluble. The semicarbazone (0·1 g.) was recrystallised from water (700 ml.) (Found : C, 36·0; H, 4·7; N, 34·7; S, 15·7. $C_6H_9ON_5S$ requires C, 36·2; H, 4·5; N, 35·2; S, 16·1%). An equal volume of saturated aqueous mercuric chloride was added to the remainder of the aldehyde solution, followed by sodium acetate to adjust the pH to 5. The mercury complex was decomposed with hydrogen sulphide. The resulting colourless solution gave positive sulphur dioxide and aldehyde tests but on concentration under reduced pressure the solution became green and finally deposited colourless crystals which almost instantaneously became Prussian-blue-coloured. It was not possible to isolate the analytically pure aldehyde.

This work was performed during the tenure by one of us (H. H.) of a British Drug Houses Research Fellowship and forms part of a thesis for the Degree of Ph.D. in the University of London.

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492. *Inhibitors of Flavoprotein Enzymes.*

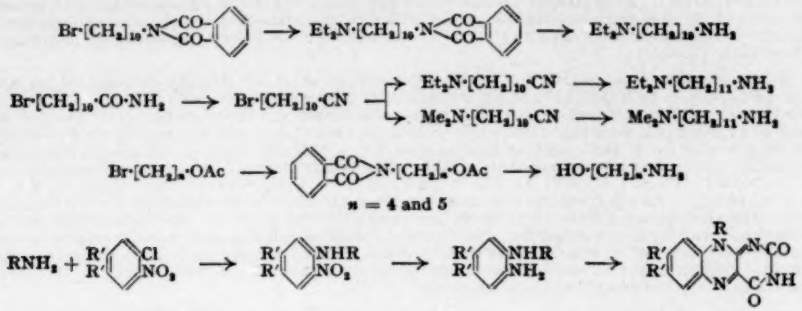
By R. B. Barlow.

Some 9-substituted 6 : 7-dichloro*iso*alloxazines and 1-substituted benziminazoles have been synthesized and tested as inhibitors of the D-amino-acid oxidase of acetone-dried pig kidney.

For several years it has been thought that molecules which have some features in common with the prosthetic group of an enzyme may inhibit it. Hellerman, Lindsay, and Bovarnick (*J. Biol. Chem.*, 1946, **163**, 553) showed that a large number of compounds, including auramine, quinine, and a number of antimalarial drugs, would inhibit the D-amino-acid oxidase of lamb kidney. The mechanism of the inhibition was not always the same—some of the compounds combined with proteins in a general way—but quinine at least appeared to compete with flavine–adenine dinucleotide (FAD) for the apoenzyme. Likewise, Singer and Kearney (*Arch. Biochem.*, 1949, **21**, 242; 1950, **27**, 348) have found that flavin analogues would inhibit the L-amino-acid oxidase of certain snake venoms. Although both enzymes have FAD as prosthetic group, the effect of a compound is not necessarily the same on each. Riboflavin, for example, is an inhibitor of the L-amino-acid oxidase but can function as prosthetic group for the D-enzyme. Dichloroflavin (Kuhn, Weygand, and Möller, *Ber.*, 1943, **76**, 1044) also inhibits the former, but is reported by Karrer and Ruckstuhl (*Bull. Schweiz. Akad. Med. Wiss.*, 1945, **1**, 236) to have no effect, even when phosphorylated, on the latter. It is remarkable, however, that both enzymes are only relatively feebly inhibited by the synthetic compounds; concentrations as high as 10^{-4}—10^{-3}M. are required.

A series of 6 : 7-dichloro-9-dialkylaminoalkyl*iso*alloxazines described in a previous paper (Barlow and Ing, *J.*, 1950, 713) had been found to inhibit the D-amino-acid oxidase of pig kidney in concentrations of this order. In place of the pyrophosphate linkage of FAD these compounds contain a basic group, and it was thought that movement of this further from the *iso*alloxazine nucleus, or replacement of it by hydroxyl, would produce more active molecules. The adenine part of FAD may also be involved in the formation of the prosthetic group–apoenzyme complex, so benziminazole and two 1-substituted benziminazoles were examined.

The compounds made are listed in Table I. They were obtained from the appropriate *o*-nitroaniline (Table II) by catalytic reduction to the *o*-phenylenediamine and treatment with alloxan or formic acid. Amino-compounds suitable for condensation with *o*-chloronitrobenzene or 2 : 4 : 5-trichloronitrobenzene were prepared by the routes shown below.



The basically substituted *iso*alloxazines were prepared from the *o*-phenylenediamines and alloxan by the action of hydrogen chloride (Kühling, *Ber.*, 1891, **24**, 2363). The exact procedure

TABLE I.

6 : 7-*Dichloroisoalloxazines.*

Prep.	9-Substituent.	M. p.† ‡	Formula.	Found, %. C.	H.	N.	Required, %. C.	H.	N.
(1) *a*	$[CH_2]_{10}\cdot NEt_2$,HCl	140°	$C_{24}H_{34}O_2N_5Cl_2,2H_2O$	50·9	6·4	—	50·9	6·7	—
(2)	$[CH_2]_{10}\cdot NEt_2$ (Picrate)	180	$C_{30}H_{36}O_9N_8Cl_2,H_2O$	49·0	5·3	14·7	48·6	5·1	15·1
(3) *a*	$[CH_2]_{11}\cdot NMe_2$ (Picrate)	180	$C_{29}H_{34}O_9N_8Cl_2,H_2O$	47·8	5·0	15·0	47·9	4·95	15·4
(4) *a*, *b*	$[CH_2]_2\cdot OH$	294—296	$C_{12}H_8O_3N_4Cl_2$	43·8	2·7	17·0	44·0	2·45	17·1
(5) *a*, *b*	$[CH_2]_3\cdot OH$	268	$C_{13}H_{10}O_3N_4Cl_2,H_2O$ §	43·4	3·4	15·5	43·5	3·3	15·6
(6) *b*	$[CH_2]_4\cdot OH$	274—276	$C_{14}H_{12}O_3N_4Cl_2$	47·3	3·3	15·8	47·3	3·4	15·8
(7) *b*	$[CH_2]_5\cdot OH$	255—256	$C_{15}H_{14}O_3N_4Cl_2$	48·9	4·0	14·9	48·8	3·6	15·2

Method: *a*, HCl–MeOH; *b*, H_3BO_3–$CH_2\cdot CO_2H$. † With decomposition.

‡ Solvents used for recrystallisation of compounds were as follows: (1) ethanol; (2), (3), and (5) aqueous alcohol; (4) ethylene glycol; (6) and (7) *n*-butanol.

§ Found: H_2O, 4·98. $C_{13}H_{10}O_3N_4Cl_2,H_2O$ requires H_2O, 5·01%.

Benziminazoles.

1-Substituent.	M. p. (or b. p.).	Cryst. from:	Formula.	Found, %. C.	H.	Required, %. C.	H.
$[CH_2]_2\cdot OH$	103° (181/0·1 mm.)	C_6H_6	$C_9H_{10}ON_2$	66·5	5·83	66·7	6·17
$[CH_2]_2\cdot OH$ (Picrate)	204	$COMe_2$	$C_{15}H_{13}O_8N_5$	46·3	3·09	46·0	3·32
$[CH_2]_3\cdot NEt_2$	(142/0·05 mm.) *	—	$C_{14}H_{21}N_3$	72·4	9·02	72·7	9·09
$[CH_2]_3\cdot NEt_2$,2HBr	176	EtOH–Et_2O	$C_{14}H_{23}N_3Br_2$	42·9	5·60	42·7	5·85

* n_D^{15} 1·5462.

TABLE II.

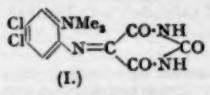
o-Nitroanilines.

Substituents in positions: 4.	5.	*N*-.	M. p.†	Formula.	Found, %. C.	H.	N.	Required, %. C.	H.	N.
(1) Cl	Cl	$[CH_2]_{10}\cdot NEt_2,HClO_4$	80°	$C_{20}H_{34}O_6N_3Cl_3,H_2O$	45·0	6·8	7·9	44·8	6·7	7·84
(2) Cl	Cl	$[CH_2]_{11}\cdot NMe_2$	40	$C_{19}H_{31}O_2N_3Cl_2$	56·6	7·5	—	56·5	7·7	—
(3) Cl	Cl	$[CH_2]_{11}\cdot NMe_2$ (Picrate)	88	$C_{25}H_{34}O_9N_6Cl_2$	47·5	5·3	—	47·4	5·4	—
(4) Cl	Cl	$[CH_2]_{11}\cdot NEt_2,HClO_4$	70	$C_{21}H_{36}O_6N_3Cl_3$	47·3	6·95	—	47·4	6·8	—
(5) Cl	Cl	$[CH_2]_2\cdot OH$	143	$C_8H_8O_3N_2Cl_2$	38·2	3·4	—	38·2	3·2	—
(6) Cl	Cl	$[CH_2]_3\cdot OH$	99	$C_9H_{10}O_3N_2Cl_2$	40·9	3·8	10·7	40·7	3·8	10·6
(7) Cl	Cl	$[CH_2]_4\cdot OH$	73	$C_{10}H_{12}O_3N_2Cl_2$	43·2	4·3	10·2	43·0	4·3	10·0
(8) Cl	Cl	$[CH_2]_5\cdot OH$	92	$C_{11}H_{14}O_3N_2Cl_2$	45·2	4·8	—	45·0	4·8	—
(9) H	H	$[CH_2]_3\cdot NEt_2$,HCl *	179—180	$C_{13}H_{22}O_2N_2Cl_2$	54·4	7·8	—	54·4	7·7	—
(10) H	H	$[CH_2]_2\cdot OH$	72	$C_8H_{10}O_3N_2$	53·0	5·4	—	52·7	5·5	—

* Obtained by Kipnis, Weiner, and Spoerri (*J. Amer. Chem. Soc.*, 1944, **66**, 1446) as picrate, m. p. 122—123°.

† Compound (2) was crystallised from petrol (b. p. 40—60°), (3) from ethanol–acetone, (5) from methanol, and the others from ethanol.

was the same as that previously described (Barlow and Ing, *loc. cit.*) but the yields of these long-chain substances were very low. No pure 6 : 7-dichloro-9-11′-diethylamino-*n*-undecyl*iso*-alloxazine could be isolated either as hydrochloride or as picrate. In several experiments a second product was formed which, in one instance, could be purified. It was less soluble in alcohol than the corresponding *iso*alloxazine and much lighter in colour, and had a completely different absorption spectrum in ultra-violet light. It may be an " anil " such as those obtained by Kühling (*Ber.*, 1893, **26**, 540), Kühling and Käselitz (*Ber.*, 1906, **39**, 1314), Kuhn and Reinemund (*Ber.*, 1934, **67**, 1932), and Tishler, Wellman, and Ladenburg (*J. Amer. Chem. Soc.*, 1945, **67**, 2165). In order to investigate this point, 4 : 5-dichloro-2-dimethylaminoaniline was treated with alloxan dissolved in acetic acid. The product was recrystallized from ethanol and had an ultra-violet absorption spectrum similar to that of the second product of the *iso*alloxazine synthesis. Unfortunately, analysis showed that the former is not the anil (I) because it contains an extra molecule of water which cannot be removed by drying.

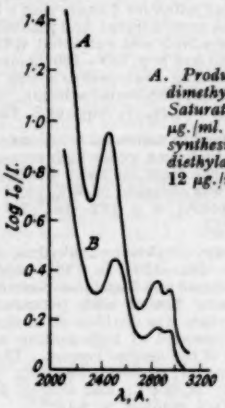


(I.)

Attempts to prepare these basically substituted *iso*alloxazines by Kuhn and Weygand's method (*Ber.*, 1935, **68**, 1282)—acetic acid as solvent, boric acid as catalyst—gave only intractable tars. The 6 : 7-di-chloro-9-ω-hydroxy-*n*-alkyl*iso*alloxazines, however, were best made by this means. The 2′-hydroxyethyl and 3′-hydroxypropyl compounds were obtained in moderate yield when the condensation was performed in methanolic hydrogen chloride, but only a very little of the 4′-hydroxybutyl and 5′-hydroxyamyl homologues could be isolated in a pure state. By the use of Kuhn and Weygand's method, these four *iso*alloxazines were obtained in excellent yield.

The synthesis of the benziminazoles presented no difficulty.

The compounds were tested on the D-amino-acid oxidase of acetone-dried pig kidney. The substrate was DL-methionine. This enzyme, unlike Singer and Kearney's L-amino-acid oxidase (*loc. cit.*), appears to be unaffected by phosphate. There was no appreciable difference in the rate of oxidation of the substrate when the phosphate buffer normally used was replaced by a borate buffer of the same pH. On the other hand, experiments suggested that the inhibitory activity of 10^{-3}M-solutions of 6 : 7-dichloro-9-5′-diethylamino-*n*-pentyl*iso*alloxazine hydrochloride (Barlow and Ing, *loc. cit.*) was slightly potentiated (25—50%) by phosphate. This effect could not be detected when the inhibitor was more dilute; 10^{-4}M-solutions inhibited the enzyme to a small but definite extent irrespective of the presence of phosphate. A similar small effect was shown by solutions of 6 : 7-dichloro-9-10′-diethylamino-*n*-decyl*iso*alloxazine hydrochloride, and of the 5′-hydroxy-*n*-amyl and 4′-hydroxy-*n*-butyl compounds, which were in the region of 10^{-4}M. This concentration is the maximum which can be achieved in these experiments for the last two, and the 3′-hydroxy-*n*-propyl and 2′-hydroxyethyl compounds are even less soluble. This low order of activity at the maximum solubility may account for Karrer and Ruckstuhl's report (*loc. cit.*) that dichloroflavin is inactive. Such a small effect would lie within the limits of error of their experiments. The inactivity of the calcium salt of dichloroflavin phosphate, which is presumably reasonably soluble in water, would seem to be indisputable.



A. Product from 4 : 5-*dichloro*-2-*dimethylaminoaniline and alloxan. Saturated solution in water,* ca. 50 *μg./ml. B. Second product in synthesis of* 6 : 7-*dichloro*-9-10′-*diethylamino*-n-*decyliso*alloxazine, 12 *μg./ml. in water.*

Benziminazole and the substituted benziminazoles were all virtually inactive at concentrations as high as 10^{-2} to 2×10^{-2}M.

From these results it appears that alteration of the nature of the 9-substituent of these 6 : 7-dichloro*iso*alloxazines makes little difference to their powers of inhibiting this D-amino-acid oxidase. Coupled with the inactivity of the benziminazoles, this suggests that FAD is held on to this particular apoenzyme mainly by the *iso*alloxazine nucleus.

Experimental.

(Analyses are by Mr. J. M. L. Cameron and Miss H. H. Kennaway. All m. p.s are uncorrected.)

Dialkylaminoalkylamines and Amino-alcohols.—10-*Bromo*-n-*decylphthalimide* was prepared by heating decamethylene dibromide (5 mols.) with potassium phthalimide (2 mols.) for 3 hours at 160—180°. Excess of dibromide was distilled off in a vacuum, and the residue shaken with water and recrystallized from ethanol. This solid was extracted in a Soxhlet apparatus with light petroleum (b. p. 40—60°). The material which separated from the petroleum in the extraction flask was filtered off and recrystallized from this solvent; it had m. p. 59° (yield 20%, based on the amount of dibromide used) (Found: N, 4·1. $C_{18}H_{24}O_2NBr$ requires N, 3·8%). The bromodecylphthalimide was heated with diethylamine (2·5 mols.) under reflux for 4 hours. Excess of diethylamine was distilled off, and the residue treated with aqueous sodium carbonate (1 mol.) and extracted with chloroform. 10-Diethylamino-*n*-decylphthalimide was obtained as an oil after removal of the chloroform and, without further purification, was decomposed with hydrazine hydrate (1 mol.) followed by excess of concentrated hydrochloric acid. The phthalylhydrazide was filtered off, and the filtrate evaporated to dryness. The residue was dissolved in a small amount of water, made strongly alkaline with solid potassium hydroxide, and extracted with benzene. The extract was dried and distilled. The yield of 10-*diethylamino*-n-*decylamine*, b. p. 157—160°/7 mm., n_D^{16} 1·4585, was 66% (Found: N, 11·8. $C_{14}H_{32}N_2$ requires N, 11·9%).

ω-Bromo-*n*-undecenoamide (Goldberg and Kelly, *J.*, 1947, 1369) was dehydrated by mixing it with an equal weight of phosphoric oxide and warming it to 140—150° in an oil-bath. If this temperature was exceeded or maintained for more than a few minutes, the yield of nitrile was appreciably reduced. The fused mass was allowed to cool, and treated with water. The resulting nitrile was extracted with a large volume of light petroleum (b. p. 40—60°), and the extract dried and distilled. The product (70%) was obtained colourless by redistillation, with b. p. 180—181°/9 mm., n_D^{18} 1·4738 (Found: C, 53·7; H, 7·8. Calc. for $C_{11}H_{20}NBr$: C, 53·7; H, 8·1%). Goldberg and Kelly (*loc. cit.*) record b. p. 158—164°/4 mm. The nitrile was heated under reflux for 4 hours with a large excess of a 33% solution of dimethylamine in alcohol. The mixture was concentrated and treated with aqueous sodium carbonate (1 mol.). The *ω-dimethylamino*-n-*undecenonitrile* was extracted with chloroform. The extract was dried and twice distilled; the product (60%) had b. p. 159—160°/9 mm., n_D^{17} 1·4500 (Found: N, 12·7. $C_{15}H_{28}N_2$ requires N, 13·3%). The corresponding *diethylamino-nitrile* was prepared in the same way with anhydrous diethylamine instead of the dimethylamine solution; it had b. p. 174°/6 mm., n_D^{14} 1·4540 (yield, 80—90%) (Found: C, 75·8; H, 12·6. $C_{15}H_{30}N_2$ requires C, 75·6; H, 12·6%).

The nitriles were dissolved in saturated solutions of ammonia in methanol and ethanol, respectively, and reduced at 120° with hydrogen under high pressure and Raney nickel catalyst. The catalyst and solvent were removed and the diamines twice distilled. 11-*Dimethylamino*-n-*undecylamine*, b. p. 156—158°/5·5 mm., n_D^{17} 1·4548, was obtained in 50% yield (Found: N, 12·8. $C_{13}H_{30}N_2$ requires N, 13·1%), and the *diethylamino*-compound, b. p. 162—164°/5·5 mm., n_D^{17} 1·4572, in 80% yield (Found: N, 11·4. $C_{15}H_{34}N_2$ requires N, 11·6%).

In exactly the same way, ethylene cyanohydrin, dissolved in methanolic ammonia, was reduced to 3-aminopropan-1-ol, b. p. 184—186°, in 75% yield. Henry (*Ber.*, 1900, **33**, 3169) records b. p. 187—188°. 4-Amino-*n*-butanol and 5-amino-*n*-pentanol were obtained from the corresponding ω-bromoalkyl acetates. These were heated with potassium phthalimide (1 mol.) at 180° for 3 hours. Unchanged bromoalkyl acetate was distilled off in steam, and the solid residue was recrystallized from ethanol. The first crop consisted of high-melting material and the phthalimidoalkyl acetates were obtained by concentration of the mother-liquors. They were recrystallized from ethanol. The yield of 4-*phthalimido*-n-*butyl acetate*, m. p. 59·5°, was 50% (Found: C, 64·2; H, 5·5; N, 5·4. $C_{14}H_{15}O_4N$ requires C, 64·3; H, 5·75; N, 5·4%), and that of 5-*phthalimido*-n-*amyl acetate*, m. p. 43°, was 33% (Found: N, 5·3. $C_{15}H_{17}O_4N$ requires N, 5·1%).

No precipitate of the phthalylhydrazide salt of the amine was formed unless these phthalimido-compounds were treated with 2 mols. of hydrazine hydrate, for the first molecule hydrolyses the acetate group. After the addition of a slight excess of concentrated hydrochloric acid and separation of the phthalylhydrazide, the filtrates were concentrated in a vacuum. They were then dissolved in water, made alkaline to litmus, and subjected to continuous extraction with benzene. The amino-alcohols are exceedingly soluble in water and not very soluble in benzene or ether. It was not feasible to precipitate them from the aqueous layer by saturating it with solid potassium hydroxide, so it was necessary to continue the extraction for several days. The yield of 4-amino-*n*-butan-1-ol, b. p. 95°/5 mm., n_D^{19} 1·4625, was 33% after 7 days (Found: C, 53·6; H, 12·6. Calc. for $C_4H_{11}ON$: C, 53·9; H, 12·4%). Henry (*loc. cit.*) records b. p. 206°/776 mm. That of 5-amino-*n*-pentan-1-ol, m. p. 29°, b. p. 107°/10 mm., n_D^{17} 1·4610, was 50% after 14 days. Putochin and Lissizin (*Ber.*, 1926, **59**, 629) record m. p. 27—28°, b. p. 221—222°, n_D^{17} 1·4618.

2-Nitro- and 4:5-Dichloro-2-nitro-N-ω-dialkylaminoalkyl- and -N-ω-hydroxyalkyl-anilines.—These *compounds* were all prepared by heating the appropriate amino-compound with a solution of *o*-chloronitrobenzene or 2:4:5-trichloronitrobenzene (10% excess) and pyridine (1 mol.) in toluene under reflux for 5—12 hours. The toluene and any unused pyridine were distilled off in a vacuum and the residue was extracted with benzene and the extract concentrated. Compounds (5), (10), and (6) (Table II) were not very soluble in this solvent and crystallized out. They were recrystallized from ethanol. The first two were a very deep red, the last was orange. The orange-coloured solutions of the remaining compounds were poured on alumina columns and washed through with benzene. The hydroxyalkyl and dimethylaminoundecyl compounds were all obtained as orange needles from benzene or alcohol but the diethylaminoalkyl compounds could only be isolated as perchlorates. They are listed in Table II together with their m. p.s and the analytical results. The yields of pure material were usually 30—40%.

The iso*Alloxazine Condensation.*—The *o*-nitroanilines were dissolved in dry methanol (basically-substituted ones which had been isolated as salt were first converted into the free base) and reduced with hydrogen and Raney nickel at room pressure and temperature. The catalyst was filtered off, and the filtrate was either treated with dry hydrogen chloride in the manner already described (Barlow and Ing, *loc. cit.*), or, if the boric acid method was to be used, concentrated in a vacuum. In the latter instance, the procedure was the same as that described by Kuhn, Weygand, and Möller (*loc. cit.*). The basically-substituted compounds were isolated from the products of the reaction in methanolic hydrogen chloride by addition of ether in the usual way. When the 6 : 7-dichloro-9-ω-hydroxyalkyl*iso*alloxazines were prepared by this method, they gradually separated from the reaction mixture and were filtered off and recrystallized. In the experiments with boric acid and acetic acid, they came out of solution during the reaction and were left, together with boric acid, when the acetic acid was distilled off. To make sure of the complete elimination of boric acid, the *iso*alloxazines were boiled with water (in which they are not very soluble) before being finally filtered off. They were dried and recrystallized. The yields of pure material were as high as 40—60% when this method was used.

The Benziminazole Condensation.—The *N*-substituted *o*-phenylenediamines, obtained as oils by reduction of the nitro-compounds in the manner described above, were boiled with anhydrous formic acid (70 ml. per 10 millimol. of compound) under reflux for 4—6 hours. The formic acid was distilled off under vacuum. The basically-substituted benziminazole was treated with ammonia solution (*d* 0·88) until alkaline, and extracted with benzene; the alcoholic compound was dissolved in methanol, heated with a trace of sodium methoxide for 1 hour on a water-bath, concentrated, diluted with water, and extracted with chloroform. The extracts were dried and twice distilled in a high vacuum. The yields of pure material were 20—30%.

The *compounds* are listed in Table I together with the analytical results, etc.

The second product formed in the preparation of the basically-substituted *iso*alloxazines was less soluble in alcohol. The compound isolated in the synthesis of the 10′-diethylamino-*n*-decyl*iso*-alloxazine was obtained crystalline by addition to the reaction mixture of a suitable amount of echer. It was recrystallized from alcohol and had m. p. (sinters) 181°, (decomp.) 206°.

The product of the reaction of alloxan with 4 : 5-dichloro-2-dimethylaminoaniline was prepared by heating equimolar quantities in glacial acetic acid 50 ml. per 10 millimol.) on a water-bath for 1 hour. The solution, which had become a deep brown, was left overnight. The acetic acid was distilled off under vacuum, and the residue recrystallized from alcohol until it was colourless (4 times). It did not melt below 300° (Found: C, 41·8; H, 3·2. $C_{12}H_{10}O_2N_4Cl_2$ requires C, 43·7; H, 3·0. $C_{12}H_{12}O_4N_4Cl_2$ requires C, 41·5; H, 3·5%). It was only very slightly soluble in water and its solutions had a greenish fluorescence. The *compound* did not lose weight when dried for 4 hours at 120°/0·5 mm.

Manometric Experiments.—The tests were performed in Warburg manometers at 37·5° with pure oxygen in the gas phase. The centre well contained 0·3 ml. of N-sodium hydroxide. The buffer (1·0 ml.; pH 7·4) and the inhibitor solution (0·4 ml.) were placed in the main compartment, and the substrate (0·4 ml. of 0·1M-DL-methionine) in the side-bulb. The enzyme (0·2 ml. of a buffered extract of the acetone-dried pig-kidney powder) was added at zero hour and gassing was begun. The flasks were equilibrated for 10 minutes before the substrate was tipped in. The time that the enzyme and inhibitor were in contact (usually 20—35 minutes) was noted but did not appear to be connected with the result. Included in each experiment were a thermobarometer and blanks containing (i) buffer and enzyme, (ii) buffer, enzyme, and inhibitor, and (iii) buffer, enzyme, and substrate only.

Results.—Effect of phosphate.

Molar concn. of phosphate:	$1·3 \times 10^{-2}$.	$1·3 \times 10^{-3}$.	$1·3 \times 10^{-4}$.	0.
Oxygen uptake after 15′ (mm.³) (duplicates) {	112	112	115	111
	106	117	112	118

Effect of inhibitors.

6 : 7-Dichloro*iso*alloxazines.

9-Substituent.	Molar concn.	Mean % inhibition after 15′.	No. of expts.
$[CH_2]_{10}$·NEt_2,HCl	10^{-4}	12	3
$[CH_2]_5$·OH	10^{-4}	16	4
$[CH_2]_4$·OH	$1·6 \times 10^{-4}$	17	4
$[CH_2]_3$·OH	5×10^{-5}	3	2
$[CH_2]_3$·OH	$1·3 \times 10^{-4}$	5	2

Benziminazoles.

1-Substituent.	Molar concn.	Mean % inhibition after 15′.	No. of expts.
H	8×10^{-3}	9	1
$[CH_2]_3$·NEt_2,2HBr	2×10^{-3}	12	2
$[CH_2]_3$·OH	2×10^{-3}	9	2

I am indebted to Professor *J.* N. Davidson and to Dr. G. Leaf for facilities for performing the manometric experiments. This work has been done during the tenure of an I.C.I. Fellowship.

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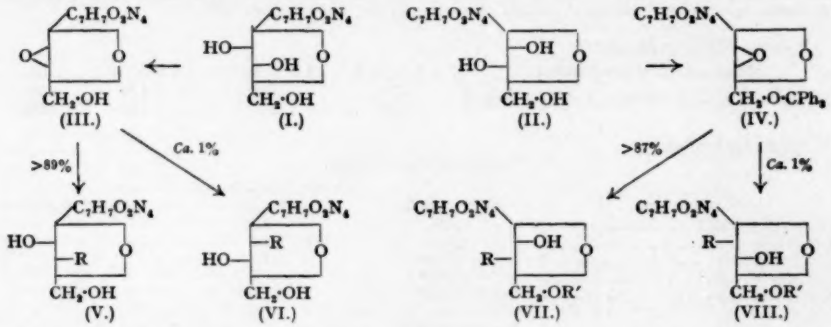
493. *Deoxyribonucleosides and Related Compounds. Part III.* Experiments with 2′ : 3′-Anhydropentofuranosyltheophyllines.*

By J. Davoll, B. Lythgoe, and S. Trippett.

The 2′ : 3′-anhydropentofuranosides (III) and (IV) have been prepared and examined in order to test the suitability of similar compounds for the synthesis of the natural 2′-deoxyribofuranosides. An orientation unfavourable to this purpose was observed when the epoxide rings were opened with sodium thioethoxide. From the products, 3′-deoxy-7-α-D-arabofuranosyltheophylline and 3′-deoxy-7-β-D-ribofuranosyltheophylline were prepared.

Recent attempts by Mukherjee and Todd (*J.*, 1947, 969) and by Kent, Stacey, and Wiggins (*J.*, 1949, 1232) to convert 2 : 3-anhydropentopyranosides into 2-deoxypentopyranosides by Prins's method (*Helv. Chim. Acta*, 1946, **29**, 371), or one of its variants, have met with only limited success on account of the unfavourable orientation observed during the opening of the epoxide ring. The nucleophilic part of the reagent molecule entered predominantly at position 3, giving derivatives from which 3-deoxypentopyranosides were obtained; the most successful experiments, carried out by Kent *et al.* with hydrobromic acid as the reagent, gave no more than 9% of the required 2-deoxypentose derivative; the reasons for this unfavourable orientation are not known.

2 : 3-Anhydropentofuranosides have not previously been accessible and so have not been investigated from this standpoint, but it was clearly desirable that this should be done, since, even if only 25%, say, of the product of the ring-opening of such a compound were a 2-deoxypentose derivative, an attractive method would be open for the synthesis of the natural 2′-deoxyribofuranosides. Recently, D-arabofuranosyl and D-xylofuranosyl derivatives of purines have become comparatively readily available (Bristow and Lythgoe, *J.*, 1949, 2306; Chang and Lythgoe, *J.*, 1950, 1992), and these compounds, which possess the *trans*-α-glycol system necessary for epoxide formation, seemed to offer the opportunity for testing this synthetic method. This has now been done, theophylline derivatives being used for convenience, and this paper reports the preparation of the epoxides (III) and (IV) and the mode of their reaction with sodium thioethoxide.



7-α-D-Arabofuranosyltheophylline (I) was converted by the usual methods into 5′-trityl and 2′ : 3′-ditoluene-*p*-sulphonyl 5′-trityl derivatives; the latter was treated with sodium methoxide, and removal of the trityl group then gave a crystalline anhydropentofuranoside whose reactions showed it to be the lyxose derivative (III). The major product of its reaction with sodium thioethoxide was the 3′-deoxy-3′-ethylthio-derivative (V; R = SEt), isolated as its crystalline 2′ : 5′-diacetate in 89% yield. This was desulphurised with Raney nickel and the acetyl groups removed, giving 3′-deoxy-7-α-D-arabofuranosyltheophylline (V; R = H), which was shown to be a 3-deoxy-sugar derivative by its failure to give the Dische diphenylamine test.

* Part II, *J.*, 1950, 1990.

The sugar liberated by hydrolysis of (V; R = H) gave on reduction an optically active 3-deoxypentitol; this must therefore be 3-deoxy-D-arabitol, which justifies the configurations assigned to the compounds (III) and (V).

It was of interest to determine how much, if any, of the 2′-deoxy-2′-ethylthioxylofuranosyl compound (VI; R = SEt) was formed along with (V; R = SEt) as a product of the ring-opening of (III). The mother-liquors from which the diacetate of (V; R = SEt) had separated were desulphurised with Raney nickel, and the product, after removal of the acetyl groups, was assayed for derivatives of 2-deoxy-sugars by the colorimetric method of Sevag, Smolens, and Lackmann (*J. Biol. Chem.*, 1940, **134**, 523). The results indicated that only about 0·8% of the anhydro-compound (III) had been opened in the desired direction.

In the hexopyranose series, removal of the toluene-*p*-sulphonyl residues from 4 : 6-benzylidene 2 : 3-ditoluene-*p*-sulphonyl methyl-α-D-altroside gives an anhydromannoside (Robertson and Whitehead, *J.*, 1940, 319) which with sodium thiomethoxide is converted into a 3-deoxy-3-methylthioaltrose derivative (Bolliger and Prins, *Helv. Chem. Acta*, 1946, **29**, 1061); the formation and ring-opening of (III) are exactly similar to these reactions. On the other hand, 2 : 3-ditoluene-*p*-sulphonyl D-glucoside derivatives give rise to 2 : 3-anhydro-D-alloside derivatives (Robertson and Griffiths, *J.*, 1935, 1193) from which 2-deoxy-2-methylthio-D-glucose derivatives are accessible in very good yield (Jeanloz, Prins, and Reichstein, *Helv. Chim. Acta*, 1946, **29**, 371). If the parallelism between the hexopyranose and pentofuranose series remarked above were general, then 2′-deoxyribofuranosides (VIII) should be accessible from the xylofuranoside (II) through the anhydroribofuranoside (IV).

The 2′ : 3′-ditoluene-*p*-sulphonyl 5′-trityl derivative of (II) did indeed give, when treated with sodium methoxide, an anhydro-compound, identified as having the ribose configuration (IV; R = CPh_3) by its reactions. This anhydro-compound did not, however, undergo ring-opening in the same direction as a 2 : 3-anhydroallopyranoside. With sodium thioethoxide the main product of reaction was 3′-deoxy-3′-ethylthio-7-β-D-xylofuranosyltheophylline 5′-trityl ether (VII; R = SEt, R′ = CPh_3), isolated crystalline in 87% yield. Successive detritylation, acetylation, desulphurisation, and deacetylation converted it into 3′-deoxy-7-β-D-xylofuranosyltheophylline (VII; R = R′ = H), which gave no colour with the Dische reagent. The sugar obtained by hydrolysis of (VII; R = R′ = H) gave optically inactive 3-deoxyxylitol when reduced, which justifies the configurations assigned to compounds (IV) and (VII). As before, the mother-liquor from which the 3′-deoxy-3′-ethylthio-compound had crystallised was treated separately so as to convert any ethylthio-compounds present into deoxy-compounds, and the product examined by the method of Sevag *et al.* The amount of 2-deoxy-sugar derivative found indicated that about 0·75% of the anhydro-compound (IV) had undergone ring-opening to give the desired 2′-deoxy-2′-ethylthio-derivative (VIII; R = SEt, R′ = CPh_3).

Preliminary experiments in which the anhydro-compound (III) was treated with lithium aluminium hydride indicate that the use of this reagent gives results no more favourable than those described above. It is clear that anhydrofuranosides similar to (III) and (IV) will be of little value for the synthesis of the natural deoxyribonucleosides until some means of reversing the orientation of their ring-opening is found.

In Part I (Davoll and Lythgoe, *J.*, 1949, 2526) the preparation of 2′-chloro D-arabopyranosyltheophylline-II and the reductive dehalogenation of 2′-chloro D-ribopyranosyltheophylline-I were described, but owing to an oversight experimental details were omitted. These are now given on p. 2233.

Experimental.

5′-Trityl 7-α-D-Arabofuranosyltheophylline.—Dry 7-α-D-arabofuranosyltheophylline (12·35 g.) and triphenylmethyl chloride (12·1 g.) were dissolved in pyridine (50 c.c.), the solution kept at 50° for 3 days and then poured into stirred ice-water (400 c.c.), and the product extracted with chloroform. The extract was washed with aqueous sodium hydrogen sulphate and with water, dried, and evaporated under reduced pressure, and the residue crystallised from a mixture of alcohol (160 c.c.) and acetone (70 c.c.). The *trityl* derivative so obtained had m. p. 215—217°, $[\alpha]_D^{15}$ −12·6° (*c*, 1·1 in chloroform) (Found: N, 9·9. $C_{31}H_{30}O_4N_4$ requires N, 10·1%).

2′ : 3′-Ditoluene-p-sulphonyl 5′-Trityl 7-α-D-Arabofuranosyltheophylline.—A solution of the above trityl compound (16 g.) and toluene-*p*-sulphonyl chloride (16·8 g.) in pyridine (75 c.c.) was kept at 50° for 4 days, then cooled to 0° and treated with water (2 c.c.). After being kept for 1 hour it was poured into ice-water (400 c.c.) and extracted with chloroform, and the chloroform extract washed, dried, and evaporated. Addition of hot alcohol (250 c.c.) to a solution of the residue in benzene (50 c.c.) caused crystallisation of the *ditoluene-p-sulphonyl trityl ether* (22·2 g.), m. p. 218°, $[\alpha]_D^{16}$ +26° (*c*, 1·3 in chloroform) (Found: C, 62·6; H, 4·9; N, 6·5. $C_{45}H_{42}O_{10}N_4S_2$ requires C, 62·6; H, 4·9; N, 6·5%).

2′ : 3′-Anhydro-7-α-D-lyxofuranosyltheophylline.—The above compound (22·2 g.), dissolved in chloroform (120 c.c.), was treated at 0° with a solution of sodium methoxide (3 g. of sodium) in methanol

(75 c.c.), and the mixture kept at 0° for 3 days and then at room temperature for a further 3 days. It was then filtered, washed with sodium hydrogen sulphate solution, dried, and evaporated under reduced pressure. The syrupy residue could not be made to crystallise, so it was hydrolysed by heating it with 80% acetic acid at 100° for 20 minutes. The filtered solution was poured into water (500 c.c.) and filtered, and the filtrate evaporated under reduced pressure; crystallisation of the residue from alcohol gave the *anhydro*-compound (2·8 g.), m. p. 204—205°, $[\alpha]_D^{18}$ −53·5° (*c*, 1·3 in pyridine) (Found: C, 49·2; H, 4·4; N, 19·2. $C_{12}H_{14}O_4N_4$ requires C, 49·1; H, 4·7; N, 19·1%).

3′-Deoxy-3′-ethylthio-7-α-D-arabofuranosyltheophylline 2′:5′-Diacetate.—A solution of the above anhydro-compound (1·5 g.) in methanol (150 c.c.) containing ethanethiol (2·1 c.c.) and sodium methoxide (0·6 g. of sodium) was heated under reflux for 2·5 hours, then cooled and neutralised with carbon dioxide. The residue left by removal of solvent under reduced pressure was extracted with hot alcohol, the filtered extract evaporated, and the residue kept overnight at 0° with pyridine (15 c.c.) and acetic anhydride (5 c.c.). The product, isolated in the usual manner, was crystallised from alcohol, giving the *diacetyl* derivative (2·02 g.), m. p. 158—160°, $[\alpha]_D^{18}$ +38·8° (*c*, 2·3 in chloroform) (Found: C, 49·5; H, 5·5 N, 13·1. $C_{18}H_{24}O_7N_4S$ requires C, 49·2; H, 5·5; N, 12·8%). The mother-liquor from which the above compound separated was evaporated and gave a resin (0·1 g.) which was desulphurised and deacetylated in the manner described below. The product, subjected to a quantitative determination by the method described by Sevag *et al.* (*loc. cit.*), was found to contain 12·2 mg. of a 2-deoxypentose, equivalent to a yield of 0·8% from the parent anhydro-compound.

3′-Deoxy-7-α-D-arabofuranosyltheophylline.—The crystalline diacetate described above (1·5 g.) was heated under reflux in alcohol (60 c.c.) with Raney nickel (8 g.) containing adsorbed hydrogen for 3·5 hours, the suspension filtered hot, and the nickel washed well with hot alcohol. Evaporation of filtrate and washings gave a resin which was kept overnight at 0° with 0·17N-methanolic barium methoxide (30 c.c.). After dilution with water (30 c.c.) most of the barium was removed as the carbonate by passage of carbon dioxide and the last traces were removed from the filtered solution with N-sulphuric acid. The solution was filtered again and evaporated under reduced pressure; crystallisation of the residue from alcohol then gave *3′-deoxy-7-α-D-arabofuranosyltheophylline*, (0·37 g.), m. p. 206—207°, $[\alpha]_D^{18}$ −1·7° (*c*, 0·6 in water) (Found: C, 48·7; H, 5·4; N, 18·8. $C_{12}H_{16}O_5N_4$ requires C, 48·7; H, 5·4; N, 18·9%). It gave no colour with the Dische reagent. A sample was hydrolysed at 100° with 0·5N-sulphuric acid, and the reaction followed polarimetrically. It was complete after 75 minutes, and a rotation of $[\alpha]_D^{18}$ −7·8° was found for the liberated 3-deoxyarabinose.

3-Deoxy-D-arabitol.—A solution of the 3′-deoxy-D-arabofuranoside (0·25 g.) in 0·5N-sulphuric acid (4 c.c.) was kept at 100° for 75 minutes and then at 0° overnight. The theophylline was filtered off, sulphuric acid removed by addition of barium hydroxide, and the filtered solution hydrogenated at 100°/100 atm. during 10 hours in the presence of Raney nickel (1 g.). After the catalyst had been removed, the solution was evaporated, and the residue crystallised from ether–alcohol, giving *3-deoxy-D-arabitol*, m. p. 102°, $[\alpha]_D^{18}$ +31° (*c*, 0·4 in water) (Found: C, 44·2; H, 8·6. $C_5H_{12}O_4$ requires C, 44·1; H, 8·8%). This compound formed a second crystalline modification, m. p. 73—75°; either form could be obtained at will by seeding a solution with the appropriate crystal.

2′:3′-Ditoluene-p-sulphonyl-5′-Trityl 7-β-D-Xylofuranosyltheophylline.—A solution of 7-β-D-xylofuranosyltheophylline (2·85 g.) and triphenylmethyl chloride (3·0 g.) in pyridine (15 c.c.) was kept at 50° for 3 days and then poured into ice-water (200 c.c.). The syrup was extracted with chloroform, the extract washed with sodium hydrogen sulphate solution and with water, and dried, and the solvent removed under reduced pressure. As the residue could not be caused to crystallise, it was dissolved in pyridine (30 c.c.), toluene-*p*-sulphonyl chloride (5·4 g.) added, and the solution kept at 50° for 4 days. It was then cooled to 0°, water (2 c.c.) added, and the whole poured into ice-water (200 c.c.). The syrup was extracted with chloroform, the extract washed, dried, and evaporated as usual, and the residue crystallised from benzene–alcohol, giving the *ditoluene-p-sulphonyl* derivative (5 g.), m. p. 195—197°, $[\alpha]_D^{18}$ +9° (*c*, 0·8 in chloroform) (Found: N, 6·7. $C_{43}H_{42}O_{10}N_4S_2$ requires N, 6·5%).

5′-Trityl 2′:3′-Anhydro-7-β-D-ribofuranosyltheophylline.—A solution of the above compound (5 g.) in chloroform (30 c.c.) was treated at 0° with methanolic sodium methoxide (20 c.c.; 0·68 g. of sodium), and the solution kept at 0° for 3 days, then at room temperature for 3 days. After the separated solid had been filtered off, the solution was washed, dried, and evaporated as usual; crystallisation of the residue from benzene–alcohol then gave the *anhydro*-compound (3·3 g.), m. p. 202—203°, $[\alpha]_D^{17}$ +23·6° (*c*, 1·6 in chloroform) (Found: C, 72·2; H, 5·8; N, 9·2. $C_{31}H_{28}O_5N_4,C_6H_6$ requires C, 72·4; H, 5·6; N, 9·1%).

5′-Trityl 3′-Deoxy-3′-ethylthio-7-β-D-xylofuranosyltheophylline.—The above anhydro-compound (3 g.) was heated under reflux with methanol in which sodium (0·65 g.) and ethanethiol (2·3 c.c.) had been dissolved. After 2·5 hours acetic acid (5 c.c.) was added to the cooled solution, which was then evaporated under reduced pressure. Crystallisation of the residue from methanol (180 c.c.) gave *5′-trityl 3′-deoxy-3′-ethylthio-7-β-D-xylofuranosyltheophylline* (2·53 g.), m. p. 186—188°, $[\alpha]_D^{17}$ +2·8° (*c*, 1·0 in chloroform) (Found: C, 66·0; H, 5·4; N, 9·4. $C_{33}H_{34}O_5N_4S$ requires C, 66·1; H, 5·7; N, 9·4%).

The mother-liquor from which this compound separated was hydrolysed and desulphurised as described below for the crystalline material, and the product, which gave a positive Dische reaction, was assayed for 2-deoxy-sugar derivatives by the method of Sevag *et al.* (*loc. cit.*). The results indicated the presence of 11 mg. of 2′-deoxyribofuranosyltheophylline, which corresponds to a yield of 0·75% on the parent anhydro-compound.

3′-Deoxy-7-β-D-ribofuranosyltheophylline.—The above crystalline ethylthio-compound (2 g.) was heated for 20 minutes at 100° with 80% acetic acid (40 c.c.), and the solution cooled, and poured into water (300 c.c.). Triphenylcarbinol was filtered off, the filtrate evaporated to dryness, and the residue dried by repeated evaporation with alcohol. It was then acetylated in the usual manner with acetic

anhydride (3 c.c.) and pyridine (10 c.c.), and the product, isolated as usual, was boiled under reflux in methanol (60 c.c.) with Raney nickel (10 g.) containing adsorbed hydrogen. After 3·5 hours the nickel was removed, and the solution evaporated to give a syrup which was deacetylated by treatment overnight at 0° with 0·167N-methanolic barium methoxide (30 c.c.). Crystallisation of the product from methanol gave the *3′-deoxyribofuranoside* (0·6 g.), m. p. 195—196°, $[\alpha]_D^{17}$ +37·8° (*c*, 0·9 in water) (Found: C, 48·4; H, 5·2; N, 18·9. $C_{12}H_{16}O_8N_4$ requires C, 48·7; H, 5·4; N, 18·9%). This material gave no colour with the Dische diphenylamine reagent. It was hydrolysed with 0·5N-sulphuric acid at 100°, and the reaction followed polarimetrically. After 75 minutes the rotation of the solution was constant, and corresponded to a value $[\alpha]_D^{18}$ −2° for the liberated 3-deoxypentose.

3-Deoxyxylitol.—A hydrolysate prepared as just described from the 3′-deoxyribofuranoside (0·4 g.) was freed from theophylline by refrigeration and filtration, and from sulphuric acid by treatment with barium hydroxide, the filtered solution was evaporated, and the residue dissolved in methanol (50 c.c.) and hydrogenated for 10 hours at 100°/100 atm. in the presence of Raney nickel catalyst (1 g.). The nickel was removed, the solution evaporated, and the residue crystallised from ether–alcohol, giving 3-deoxyxylitol, m. p. 66—67°, $[\alpha]_D^{18}$ ±0° (cf. Kent *et al.*, *loc. cit.*) (Found: C, 43·9; H, 9·0. Calc. for $C_5H_{12}O_4$: C, 44·1; H, 8·8%).

2′-Chloro D-*Arabopyranosyltheophylline*-II.—The corresponding 3′ : 4′-diacetate (0·36 g.) (Davoll and Lythgoe, *loc. cit.*) was kept at 0° for 3 days with methanolic ammonia (50 c.c., saturated at 0°), solvents removed under reduced pressure, and the residue crystallised from water, giving the *2′-chloro* D-*arabinoside* (0·2 g.) as needles which sintered at 140°, m. p. 200°, $[\alpha]_D^{14}$ +68° (*c*, 0·69 in water) (Found: C, 43·4; H, 4·5; N, 17·0. $C_{12}H_{15}O_8N_4Cl$ requires C, 43·6; H, 4·5; N, 16·9%).

Reductive Dehalogenation of 2′-Chloro D-*Ribopyranosyltheophylline*-I.—The 2′-chloro D-riboside (0·46 g.), dissolved in 0·04N-sodium hydroxide (52 c.c.), was hydrogenated at room temperature and pressure in the presence of palladised barium sulphate (5%; 1 g.). The calculated volume of hydrogen was absorbed in 5 hours, and the filtered solution was neutralised with hydrochloric acid and evaporated under reduced pressure. The residue was treated with pyridine and acetic anhydride, and the product isolated in the usual manner. Crystallisation from alcohol gave 3′ : 4′-diacetyl 2′-deoxy-D-ribopyranosyltheophylline-I (0·3 g.), identical in m. p. with material prepared as previously described.

We thank Professor A. R. Todd, F.R.S., for his interest in this work, and the Department of Scientific and Industrial Research for a Senior Assistant's Grant (to J. D.).

UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, April 30th*, 1951.]

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494. *Ion Association in Aqueous Solutions of Metal Dicarboxylates.*

By J. M. PEACOCK and J. C. JAMES.

The ion association which occurs between cations and dicarboxylate anions in aqueous solution has been studied by potentiometric, conductometric, and spectrophotometric methods. Dissociation constants are reported for the products of such association, and the significance of these results is discussed.

AQUEOUS solutions in which metal cations and dicarboxylate anions are present often show marked specific interaction effects which can satisfactorily be accounted for in terms of mass-action dissociation constants. In some cases the resultant complexes behave as abnormally weak electrolytes, a typical example being copper malonate, which has been shown by Ives and Riley (*J.*, 1931, 1998) to be a considerably weaker electrolyte than acetic acid. The present investigation provides further information as to the effects of structural factors and on the nature of the linkages involved between cations and dicarboxylate anions. Solutions containing cations M [where M = $Ba^{\cdot\cdot}$, $Cu^{\cdot\cdot}$, $La^{\cdot\cdot\cdot}$, or $Co(NH_3)_6^{\cdot\cdot\cdot}$] and anions A, from dicarboxylic acids of the series $[CH_2]_x(CO_2H)_2$ have been studied by potentiometric, conductometric, and spectrophotometric methods; less detailed measurements for other dicarboxylic acids are also reported.

Potentiometric Measurements.—In dicarboxylic acid buffer solutions ionic concentrations are governed by the equilibria $H_2A \rightleftharpoons H^{\cdot} + HA'$, $HA' \rightleftharpoons H^{\cdot} + A''$. On addition of a bivalent metal chloride, these equilibria are displaced and a change in the hydrogen-ion concentration occurs, primarily because of the reaction $M^{\cdot\cdot} + HA' \rightleftharpoons MA + H^{\cdot}$. Such pH changes are often large, and the effect has been used by a number of workers (*e.g.*, Cannan and Kibrick, *J. Amer. Chem. Soc.*, 1938, **60**, 2314; Stock and Davies, *J.*, 1949, 1371) for the determination of the concentrations of the various ionic species present, and thence for the calculation of dissociation constants. This method has been used in the present work, the

procedure adopted being to determine the pH's of solutions of concentration (*a*) 0·005M-NaHA, 0·005M-MCl_3 (or MCl_3) and (*b*) 0·005M-NaHA, 0·015M-MCl_3 (or MCl_3) by means of the glass electrode. pH has been taken as defined by $pH = -\log f_1[H^+]$, where f_1 is the mean activity coefficient for univalent ions. The calculation method was essentially that of Stock and Davies (*loc. cit.*), the concentration of undissolved metal dicarboxylate being obtained as follows. The ionic species present are: H^+, Na^+, Cl', M^{++}, MA, A'', HA', H_2A, and OH' (metal chlorides and sodium dicarboxylates are known to be highly dissociated. On general grounds it appears unlikely that other ionic species such as $MHMal^+$ are present in appreciable concentrations). Then the concentrations of Na^+ and Cl' are known, that of OH' is negligible in acid solution, and the remaining concentrations can be calculated from (i) the measured $pf[H^+]$ value, (ii) the electroneutrality equation, (iii) the known total concentration of bivalent metal, and (iv) and (v) the pK_1 and pK_2 values for the dicarboxylic acid. For tervalent cations M^{+++}, the concentration of intermediate ion MA^+ is found in a similar way. Ionic strengths I can then be calculated from $I = \frac{1}{2}\Sigma m_i z_i^2$, where m_i is the molar concentration of ions of type i and valency z_i.

Typical results are given for illustration purposes in Table I.

TABLE I.

Ion association in malonate solutions.

Concentration (millimols./l.)	$pf_1[H^+]$	H_2Mal	Mal''	La^{+++}	$LaMal^+$	$HMal'$	I	$K \times 10^4$
0·005M-$LaCl_3$ + 0·005M-NaHMal	3·36	0·823	0·0256	4·01	0·992	3·17	31·176	1·4
0·015M-$LaCl_3$ + 0·005M-NaHMal	3·16	1·15	0·0195	13·9	1·09	2·8	92·890	1·2

Dissociation constants have been calculated from the equations $K = [M^{++}][A'']f_\pm^2/[MA]$ for bivalent cations and $K = [M^{+++}][A''] \cdot f_{M^{+++}}f_{A''}/[MA^+]f_{MA^+}$ for tervalent cations, and are summarised in Table II. The ionic strengths of the solutions used in the present work were too great for the limiting form of the Debye–Hückel equation to be applied, and the empirical extension due to Davies (*J.*, 1938, 2093) has therefore been used for calculation of the activity coefficients.

TABLE II.

Dissociation constants ($K \times 10^4$) *of metal dicarboxylates.*

	CuX	LaX^+	$Co(NH_3)_4X^+$		CuX	LaX^+	$Co(NH_3)_4X^+$
Malonate	0·0144	0·13	2·9	Phthalate	0·86	0·18	1·8
Succinate	4·7	1·1	11·5	Maleate	1·25	0·28	0·61
Glutarate	6·9	1·5	6·7	Fumarate	31	9·8	43
Adipate	4·2	0·8	4·5				

In the above treatment no account has been taken of further association to give complex ions of the type M_2A^{++} and MA_2''. This assumption, which has also been made by previous workers [*e.g.*, Stock and Davies (*loc. cit.*)], appears to be justified at the low concentrations studied.

Conductivity Measurements.—Copper malonate. Salts with abnormally low dissociation constants often show a marked tendency to auto-complex formation, examples being ferrous oxalate and nickel oxalate solutions, for which the conductivities provide evidence of complex formation even in very dilute solution. As copper malonate also appears to be an abnormally weak salt, the use of a potentiometric method neglecting auto-complex formation might not be justifiable here. For this reason, the dissociation constant for copper malonate has also been determined directly from conductivity measurements on dilute aqueous solutions. The results are tabulated in Table III.

TABLE III.

Conductivities of copper malonate solutions at 25° (*c* is given in g.-equivs./l.).

10^4c	Λ	$10^6K'$	10^4c	Λ	$10^6K'$	10^4c	Λ	$10^6K'$	10^4c	Λ	$10^6K'$
	Run 1. $K_{H_2O} = 2·92 \times 10^{-7}$.						Run 2. $K_{H_2O} = 5·16 \times 10^{-7}$.				
3·5562	11·57	$1·80_5$	12·835	7·03	2·25	2·4573	13·46	1·73	9·3800	7·78	$2·04_5$
6·0796	9·28	$1·93_3$	15·972	6·54	2·41	4·3379	10·54	1·80	13·327	6·86	2·22
9·4586	7·84	$2·09_5$	18·594	6·25	2·54	6·9210	8·74	1·93	18·041	6·22	2·51

At 25°, combination of the value $\Lambda_0(Cu^{++}) = 53·6$ given by Owen and Gurry (*J. Amer. Chem. Soc.*, 1938, **60**, 3074) for the copper ion with Jeffrey and Vogel's (*J.*, 1935, 21) value of 64·7 for

the malonate ion leads to $\Lambda_0 = 118{\cdot}3$ for the conductivity at infinite dilution. The limiting Onsager and Debye–Hückel equations then become

$$\Lambda = 118{\cdot}3 - 228{\cdot}6I^{\frac{1}{2}} \text{ and } -\log f_{\pm}^2 = 4{\cdot}074I^{\frac{1}{2}}$$

The ionic strength is given by $I = 2\alpha c$, where c is the concentration in g.-equivs./l. Values for α, the degree of dissociation, have been calculated, and dissociation constants, K', have then been calculated for each point from the equation

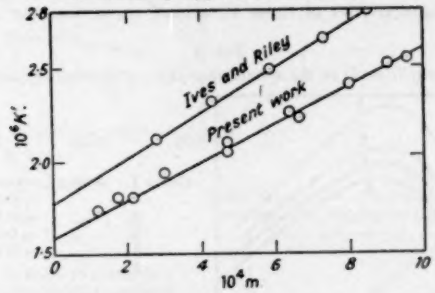
$$\log K' = -4{\cdot}074I^{\frac{1}{2}} + \log [\alpha^2 c/2(1-\alpha)]$$

Values of K' are given in Table III, and have been plotted against c in Fig. 1; a steady increase in K' with increase in concentration occurs, presumably because of further association to give complex ions Cu_2Mal^{++} and $CuMal_2''$. A short linear extrapolation to zero concentration gives $K = 1{\cdot}59 \times 10^{-6}$ for the true dissociation constant of copper malonate.

Ives and Riley (*loc. cit.*) obtained the value $K = 2{\cdot}5 \times 10^{-6}$ from conductivity measurements; these workers, however, used the value $\Lambda_0 = 113{\cdot}8$ as a basis for calculation. Recalculation of this result, Λ_0 being taken as 118·3, gave results which have been plotted in Fig. 1, and lead

FIG. 1.

The dissociation of copper malonate solutions.



to the value $K = 1{\cdot}77 \times 10^{-6}$ on extrapolation to zero concentration. The potentiometric method gives $K = 1{\cdot}4_4 \times 10^{-6}$, and although the agreement with the conductivity method may be to some extent caused by a compensation of errors, it would appear that the primary dissociation constants derived from this method are not greatly in error, and may safely be used for the purpose of making a general survey of the extent of dissociation of metal dicarboxylates.

Hexamminocobaltic oxalate. The dissociation of this salt has been studied by conductivity measurements on very dilute aqueous solutions, since the solubility (0·383 millimol./l.) is too low for convenient application of the potentiometric method. The results are set out in Table IV.

TABLE IV.

Conductivities of hexamminocobaltic oxalate solutions at 25° (c in g.-equivs./l.).

	(c in g.-equivs./l.)						
10^4c	4·5960	5·3031	5·9578	9·1920	10·213	13·788	9·722
Λ	134·0	132·3	128·9	123·3	120·4	114·7	121·2
$K \times 10^4$	4·58	4·97	4·73	4·92	5·44	5·64	5·38

The conductivity at infinite dilution has been taken to be 173·3, from the values $\Lambda_0[Co(NH_3)_6^{***}] = 99{\cdot}2$ (Jenkins and Monk, *J.*, 1951, 68) and $\Lambda_0(C_2O_4'') = 74{\cdot}1$ (Darken, *J. Amer. Chem. Soc.*, 1940, **62**, 846). Marked deviations from the limiting Onsager equation occur even in very dilute solution, as is to be expected with a salt of this valency type. The extent of such association has been estimated by the method described by Jenkins and Monk (*ibid.*, 1950, **72**, 2695) for the similar 3—2-valency type electrolyte lanthanum sulphate. In the present case, deviations from the limiting Onsager equation have been ascribed to formation of the intermediate ion $[Co(NH_3)_6][C_2O_4]^+$, the mobility for this ion being taken as $\frac{1}{3} \times 99{\cdot}2 = 33$. Values derived for the dissociation constant of this ion are given in Table IV. The drift in K values does not appear to be due to auto-complex formation, since solubility measurements (James, unpublished work) give no indication of such tendencies, but is probably the result of

the approximations necessarily made in the calculation. Extrapolation to zero concentration gives the value $4{\cdot}0 \times 10^{-4}$ for K.

Barium dicarboxylates. As these salts are too extensively dissociated in aqueous solution for the potentiometric method to be applied, recourse has been had to the conductometric method

TABLE V.

The dissociation of barium dicarboxylates (c in g.-equivs./l.).

10^3c.	A.	10^3K.	10^3c.	A.	10^3K.	10^3c.	A.	10^3K.	10^3c.	A.	10^3K.
Barium malonate.			Barium succinate.			Barium glutarate.			Barium adipate.		
1·551	110·6	8·5	1·515	107·6	9·0	1·776	102·1	8·1	1·385	101·1	10
1·899	107·7	7·2	1·855	105·7	8·8	2·088	102·4	12	1·995	100·1	15
2·234	105·2	6·5	2·144	102·9	7·2	2·696	97·5	7·5	2·559	97·2	12

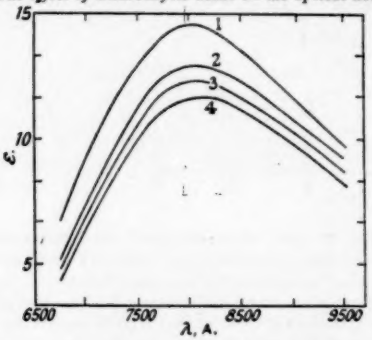
Mean values for K:

	Oxalate.	Malonate.	Succinate.	Glutarate.	Adipate.
Barium	0·0047	0·0074	0·0083	0·0092	0·012

described by Topp and Davies (*J.*, 1940, 87). Conductivity titrations of dicarboxylic acids have been carried out with standard baryta, rigorous precautions being taken to exclude atmospheric carbon dioxide. The conductivities for the barium salts of the acids were obtained from the end-point conductivities of these titrations, corrections for hydrolysis and solvent

FIG. 2.

The effect of dicarboxylic acids on the optical absorption of aqueous copper sulphate solutions.



Curve 1. 0·06M-*Copper sulphate*
2. ,, +0·10M-*malonic acid.*
3. ,, +0·10M-*succinic acid.*
3. ,, +0·10M-*glutaric acid.*
4. ,, +0·10M-*adipic acid.*

conductivity being made by Jeffery and Vogel's method (*loc. cit.*). Conductivities at infinite dilution were obtained by combining Shedlovsky and Brown's value (*J. Amer. Chem. Soc.*, 1934, **56**, 1066) of $\Lambda_0(Ba^{++}) = 63{\cdot}64$ with Jeffery and Vogel's (*loc. cit.*) values for the dicarboxylate ions. Results are given in Table V, values for the degree of dissociation α and the dissociation constant K having been calculated for each point by Topp and Davies's method (*loc. cit.*).

Topp and Davies found $K = 0{\cdot}027$ for barium succinate and $K = 0{\cdot}014$ for barium adipate. The difference between their value and our own would appear to be due to our use of Jeffery and Vogel's values for dicarboxylate ionic mobilities at infinite dilution. Recalculation of Topp and Davies's results on this basis gives $K = 0{\cdot}011$ for barium succinate and $K = 0{\cdot}014$ for barium adipate, in good agreement with our own values.

Spectrophotometric Measurements.—Cupric ion. It has been known for some time that the intensity and wave-length of the cupric-ion absorption peak are markedly displaced in solutions containing anions of organic acids, and use has been made of these effects in the investigation of the formulæ and stability of copper complexes; *e.g.*, spectrophotometric methods have been used by Bobtelsky and Jordan (*J. Amer. Chem. Soc.*, 1945, **67**, 1824) to investigate citrate and tartrate complexes, and by Klotz, Faller, and Urquhart (*J. Phys. Chem.*, 1950, **54**, 18) with copper–protein complexes. It seemed of interest to extend such studies to the cupric salts of dicarboxylic acids, for which no data appear to be available.

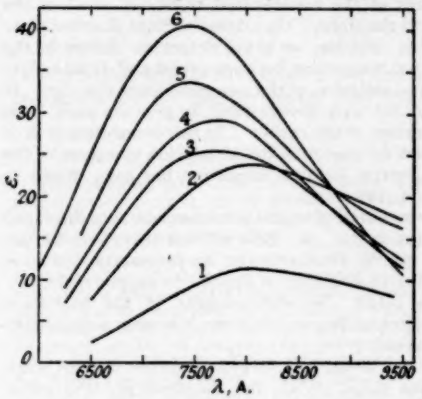
In Fig. 2 absorption curves are given for 0·06M-copper sulphate solution and for mixtures with dicarboxylic acids. An appreciable increase in the extent of absorption occurs with malonic acid and, to a lesser extent, with succinic, glutaric, and adipic acids. Similar increases

were observed with maleic, fumaric, phthalic, and tartaric acids. In Fig. 3 absorption curves are given for solutions containing equimolecular amounts of copper sulphate and the sodium dicarboxylate; the changes which occur are far more marked than in Fig. 2, as is to be expected if the dicarboxylate group must be ionised for complex formation to occur.

Copper succinate, glutarate, and adipate were too insoluble in neutral solution for the absorptions to be studied.

FIG. 3.

The optical absorption of copper sulphate–sodium dicarboxylate solutions.



Curve 1. 0·02M-*Copper sulphate.*
2. ,, +0·02M-*sodium tartrate*, pH 5·42.
3. ,, +0·02M-*sodium malonate*, pH 4·09.
4. ,, +0·02M-*sodium malate*, pH 4·04.
5. ,, +0·02M-*sodium phthalate*, pH 5·70.
6. ,, +0·02M-*sodium citrate*, pH 4·00.

FIG. 4.

Optical absorption of solutions containing 0·02M-copper sulphate and varying amounts of sodium malate.

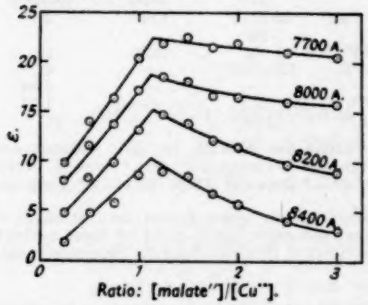
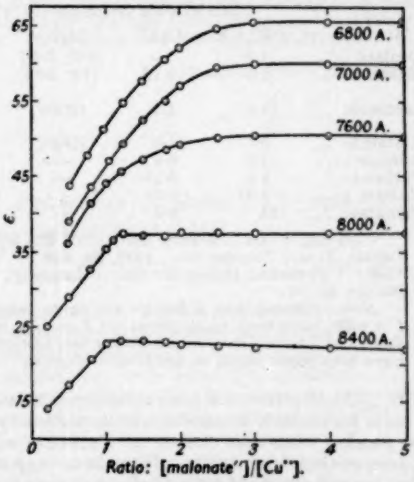


FIG. 5.

Optical absorption of solutions containing 0·02M-copper sulphate and varying amounts of sodium malonate.



The absorptions of solutions containing 0·02 g.-mol./l. of copper sulphate and varying amounts of sodium malate have been studied, and in Fig. 4 optical extinctions have been plotted against the mol. ratio [malate'']/[Cu''] for a series of different wave-lengths in the absorption band. At each wave-length a definite break is obtained at a point corresponding to reaction in the mol. ratio of 1 : 1. Copper lactate was found to behave in the same way, although the lower complex stability causes less sharp breaks in the extinction curves. Bobtelsky and Jordan (*loc. cit.*) report similar results for copper and nickel citrates and for nickel tartrate.

The results of similar measurements on solutions containing copper sulphate and sodium malonate are given in Fig. 5. The results are less satisfactory in this case, as the location of the intercept varies with wave-length, indicating that at least two absorbing species are formed, corresponding probably to CuMal and $CuMal_2''$. With the corresponding copper phthalate system, although marked absorption changes occurred, no definite points of inflection could be detected in the curves, presumably because of the lower stability of the complex.

Hexamminocobaltic ion. In aqueous solution the hexamminocobaltic ion shows absorption maxima at 4714 A. and 3376 A. (Tsuchida, *Bull. Chem. Soc. Japan*, 1938, **13**, 5), the first band being generally attributed to electron transitions in the unsaturated transition shell of the central atom and the second band to co-ordination electrons. On adding sodium dicarboxylate solutions to 0·01M-solutions of hexamminocobaltic chloride, we could detect no change in the intensity and location of these bands, indicating that interaction between cation and dicarboylate anion was not marked enough to disturb the optical electrons of the hexamminocobaltic ion. It is probable that any association which may occur is purely electrostatic, to give ion pairs, as is to be expected from the stable electronic configuration of the cation. In this connection it is of interest that with lanthanum ferricyanide solutions no change in the absorption spectrum of the ferricyanide ion could be detected, although extensive ion-pair formation has been shown to occur (James and Davies, *Proc. Roy. Soc.*, 1948, *A*, **195**, 116).

Polarographic Measurements.—The half-wave potential of cupric ion is considerably displaced in presence of citrate ion, and Bobtelsky and Jordan (*loc. cit.*) have utilised this effect for the determination of the dissociation constant of cupric citrate by an amperometric titration method. As it appeared possible that a similar method might be applied to copper malonate, exploratory polarographic measurements were made. No displacement in the half-wave potential for cupric ion could be detected in copper malonate solutions, however, presumably because the complex stability was insufficiently great.

Discussion.—Dissociation constants obtained for metal dicarboxylates in the present work are given in Table VI, together with previous data. With the dicarboxylic acid series

TABLE VI.

Dissociation constants ($K \times 10^4$) *of metal dicarboxylates.*

	$Co(NH_3)_4X^+$	LaX^+	MgX	CaX	BaX	ZnX	CuX
Oxalate	4·0	—	(3·7[1], 5·6[2])	(10[3])	(47[3])	(0·13[3], 0·099[4])	(0·007[5])
Malonate ...	2·9	0·13	(14[6], 25[3])	(32[6], 69[3])	74 (196[6], 117[3])	(1·79[7], 2·1[8], 3·3[3])	0·0159 (0·018[9])
Succinate ...	11·5	1·1	(126[3])	(40[9], 126[3])	83 (110°, 186[3])	(33[3])	4·7
Glutarate ...	6·7	1·5	(166[3])	(174[3])	92	(50[3])	6·9
Adipate	4·5	0·8	—	(64[9])	120 (140[9])	—	4·2
Phthalate ...	1·8	0·18	—	—	—	—	0·86
Maleate	0·61	0·28	—	(37[9])	(55[9])	—	1·25
Fumarate ...	43	9·8	—	(100[9])	(260[9])	—	31

[1] Davies, *Trans. Faraday Soc.*, 1927, **23**, 351. [2] Cannan and Kibrick, *loc. cit.* [3] Money and Davies, *Trans. Faraday Soc.*, 1932, **28**, 609. [4] Vosburgh and Beckman, *loc. cit.* [5] Ives, *J.*, 1933, 1360. [6] Stock and Davies, *loc. cit.* [7] James, *J.*, 1951, 153. [8] Ives and Riley, *loc. cit.* [9] Topp and Davies, *loc. cit.*

Note.—Cannan and Kibrick's constants, which were originally given for an ionic strength of $I = 0·20$, have been recalculated for $I = 0$ by using the conversion factor given by these authors. Topp and Davies's data for succinates and adipates, and those of Ives and Riley for copper malonate have been recalculated as described previously.

$[CH_2]_x(CO_2H)_2$ there is a general tendency for dicarboxylate stability to decrease as x increases. This is particularly noticeable with transition-type metal ions, a 500-fold increase in K occurring on passing from zinc oxalate to glutarate, whereas only a 2-fold increase occurs with the corresponding barium salts. It has been suggested by Jones, Monk, and Davies (*J.*, 1949, 2693) that the magnitude of a dissociation constant is dependent mainly on two factors, the hydration energy of the cation and the energy of interaction of the associating ions. It seems probable that with cations such as magnesium, barium, lanthanum, and hexamminocobalt relatively little chelation occurs, and that the interaction energy is largely of an electrostatic nature. Transition-metal ions have much stronger chelating tendencies, and the stabilities of their dicarboxylates will in consequence be far more markedly dependent on ring size.

Abnormally weak salts usually form auto-complexes readily, as is shown by Vosburgh and Beckman's solubility measurements (*J. Amer. Chem. Soc.*, 1940, **62**, 1028) for zinc and cadmium

oxalates and by Ives and Riley's conductivity measurements (*loc. cit.*) for copper malonate. The dicarboxylates of magnesium, calcium, barium, and hexamminocobalt are much stronger salts, and solubility measurements show that no appreciable tendency to auto-complex formation exists (Money and Davies, *J.*, 1934, 400; James, unpublished work).

Maleates and fumarates show differences in K which are to be expected on steric grounds, the variation being especially marked with tervalent ions, which can chelate with maleate but not with fumarate ions, as can be seen from the following dissociation constant ratios for fumarates and maleates:

Cation	$Ca^{"}$	$Ba^{"}$	$Co(NH_3)_6^{'''}$	$La^{'''}$	$Cu^{"}$
$K_{fumarate}/K_{maleate}$	2·70	4·7	70	35	25

Phthalates appear to occupy a position intermediate in strength between malonates and succinates, and are slightly weaker than maleates.

Experimental.

Hexamminocobaltic chloride and oxalate were prepared and purified by the method of Bjerrum and Reynolds ("Inorganic Syntheses," McGraw-Hill, 1946, Vol. II, Chap. 8), and copper malonate by the method of Ives and Riley (*loc. cit.*). Other materials were Kahlbaum or AnalaR samples, and were used without further purification.

Measurements of pH were made at 25° by the glass-electrode method, Smith and Speakman's "reproducible liquid junction" apparatus (*Trans. Faraday Soc.*, 1948, **44**, 1031) being used in conjunction with a Marconi bench-model pH meter. Standardisation was effected with suitable buffer solutions, as recommended by Hitchcock and Taylor (*J. Amer. Chem. Soc.*, 1937, **59**, 1812). Conductivity measurements on copper malonate and hexamminocobaltic oxalate solutions were carried out with equipment similar to that described previously (Davies, *J.*, 1937, 432; James and Davies, *Proc. Roy. Soc.*, 1948, *A*, **195**, 116). The quartz cell used was of the Hartley and Barrett type, and was calibrated by Davies's method (*Trans. Faraday Soc.*, 1929, **25**, 129). Concentrations of copper malonate stock solutions were determined gravimetrically. Hexamminocobaltic oxalate solutions of known concentration were prepared by dilution of a saturated solution at 25°, the solubility of the salt having been determined by the method of Money and Davies (*loc. cit.*). Conductivities for barium salts of dicarboxylic acids were obtained by using the titration method described by Topp and Davies (*J.*, 1940, 87). Optical absorption measurements were made with a Unicam spectrophotometer and 1-cm. quartz cells. Exploratory polarographic measurements were made by means of manual apparatus of the type described by Kolthoff and Lingane ("Polarography," Interscience Publishers Inc., N.Y., 1946, p. 215).

The University, Glasgow. [*Received, March 28th*, 1951.]

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495. *The Preparation and Properties of Some* β-(2- *and* 4-*Aroylphenoxy*)*propionic Acids.*

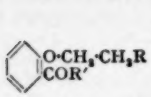
By Elizabeth M. D. Simpson, Muriel L. Tomlinson, and (in part) Helan V. Taylor.

β-(4-Benzoyl-3-hydroxyphenoxy)propionic acid, β-(4-benzoyl-3-methoxyphenoxy)propionic acid, β-(2-benzoyl-5-methoxyphenoxy)propionic acid, and the corresponding 2- and 4-veratroyl compounds have been obtained by interaction of benzoyl and veratroyl chlorides with ethyl β-*m*-methoxyphenoxypropionate in the presence of aluminium chloride. It has been found that these substances are readily dissociated by alkali with the removal of the $-CH_2{\cdot}CH_2{\cdot}CO_2H$ group and the production of the corresponding phenol. Similar Friedel–Crafts reactions using β-*m*-methoxyphenoxypropionitrile, *m*-di-2′-cyanoethoxybenzene, and *m*-di-2′-carbomethoxyethoxybenzene have also been carried out.

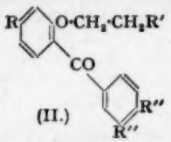
We were interested in the possibility of preparing 1:2-benzopyrans by the cyclisation of compounds of the type (I; R = CN or CO_2Et). Previous work having shown that it is difficult to bring about a reaction between acrylonitrile and a phenolic hydroxyl group that is *ortho* to a carbonyl group (Bachman and Levine, *J. Amer. Chem. Soc.*, 1948, **70**, 599; Taylor and Tomlinson, *J.*, 1950, 2724), we decided to investigate the possibility of introducing aroyl groups

into β-*m*-methoxyphenoxypropionitrile and the corresponding ethyl ester under Friedel–Crafts conditions, as an alternative route for the production of substances of the type (I; R = CN or CO_2Et).

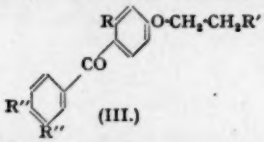
Ray, Silooja, and Wadha (*J. Indian Chem. Soc.*, **1933**, **10**, 617) were unsuccessful in their attempt to prepare β-(5-methoxy-2-veratroylphenoxy)propionate (II; R = R″ = OMe, R′ =



(I.)



(II.)



(III.)

CO_2Et) from veratroyl chloride and ethyl β-*m*-methoxyphenoxypropionate: they isolated 2-hydroxy-4 : 3′ : 4′-trimethoxybenzophenone and a small quantity of a substance, m. p. 175°, which they decided was probably 4-hydroxy-2 : 3′ : 4′-trimethoxybenzophenone. They concluded that the propionic ester residue had been extruded during the Friedel–Crafts reaction to form these compounds.

We began our investigation by using β-*m*-methoxyphenoxypropionitrile, and benzoyl chloride with aluminium chloride in nitrobenzene solution, and from the resulting product obtained β-(4-benzoyl-3-hydroxyphenoxy)propionitrile (III; R = OH, R′ = CN, R″ = H) and a non-phenolic residue which failed to crystallise but afforded β-(2-benzoyl-5-methoxyphenoxy)-propionic acid (II; R = OMe, R′ = CO_2H, R″ = H) on hydrolysis with acid. In a similar reaction with ethyl β-*m*-methoxyphenoxypropionate in the place of the nitrile, the product was found to contain carboxylic, phenolic, and neutral fractions. The acid, removed with sodium carbonate, was β-(2-benzoyl-5-methoxyphenoxy)propionic acid (II; R = OMe, R′ = CO_2H, R″ = H), and the phenolic fraction, extracted with cold dilute sodium hydroxide, afforded β-(4-benzoyl-3-hydroxyphenoxy)propionic acid (III; R = OH, R′ = CO_2H, R″ = H), the ester grouping having been hydrolysed during the extraction. From the neutral part of the reaction product there was isolated, after hydrolysis with dilute acid and careful fractional crystallisation a further quantity of β-(2-benzoyl-5-methoxyphenoxy)propionic acid together with some β-(4-benzoyl-3-methoxyphenoxy)propionic acid (III; R = OMe, R′ = CO_2H, R″ = H).

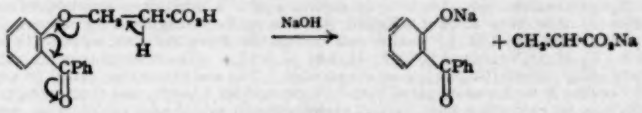
It seemed worth while, in view of these results, to repeat the reaction with veratroyl chloride and ethyl β-*m*-methoxyphenoxypropionate, and in this way we obtained a series of compounds completely analogous to those described above, namely, β-(3-hydroxy-4-veratroylphenoxy)-propionic acid (III; R = OH, R′ = CO_2H, R″ = OMe), β-(3-methoxy-4-veratroylphenoxy)-propionic acid (III; R = R″ = OMe, R′ = CO_2H), and β-(5-methoxy-2-veratroylphenoxy)-propionic acid (II; R = R″ = OMe, R′ = CO_2H). The reaction mixture from a similar experiment with veratroyl chloride and β-*m*-methoxyphenoxypropionitrile gave neutral and phenolic fractions. The former must have contained the isomers (II and III; R = R″ = OMe, R′ = CN), and the latter (III; R = OH, R′ = CN, R″ = OMe). Both fractions, however, failed to crystallise and were investigated by degradation with alkali (see later), and by acid hydrolysis which always gave very poor yields of the expected acids, probably because some demethylation occurs.

Hence, in all these reactions it is clear that the aroyl group is introduced, as would be expected, partly *ortho* to the cyanoethoxy- or carbethoxyethoxy-groups and partly *ortho* to the methoxyl group; the latter may be partly or completely extruded during the reaction but no compound has been isolated that makes it certain that the other substituent is ever removed in this way. All the isolated compounds that had lost this group had been in contact with alkali, which it has been found (see later) effects that kind of decomposition with great ease.

Both possible positions of substitution seem to be attacked in comparable proportions, but it is difficult to estimate these proportions with any accuracy as in no case could all the products be directly crystallised, and in all instances there was a considerable quantity of material that remained as a gummy mass.

It has been found, with substances of the types (II) and (III), that alkali, even under quite mild conditions, removes the cyanoethoxy- and carboxyethoxy-group with the formation of a phenol and presumably acrylonitrile or acrylic acid. This is comparable with the ring opening that occurs with flavanones under the influence of alkali to give *o*-hydroxychalkones, but here, as the group is separated from the rest of the molecule, the reaction is irreversible. This reaction no doubt explains Ray, Silooja, and Wadha's failure (*loc. cit.*) to isolate any β-phenoxypropionic

acids in their investigation, as they hydrolysed their crude ester product with alcoholic alkali which would readily dissociate such substances:



This dissociation also causes considerable loss of materials even with the cold dilute alkali extraction, followed by rapid acidification, that we employed. During the extraction, decomposition products could always be detected by smell, and the product extracted by alkali in the reaction between veratroyl chloride and β-*m*-methoxyphenoxypropionitrile appears to have undergone extensive degradation: β-(5-methoxy-2-aroyl)propionic acids are decomposed rapidly too.

The constitutions allotted to the substances described above are based on the following observations. β-(4-Benzoyl-3-hydroxyphenoxy)propionic acid and the corresponding nitrile gave a violet colour with ferric chloride and a deep yellow solution in sodium hydroxide, and therefore the OH and CO groups must be *ortho* to one another in these compounds. This nitrile could be hydrolysed to the above acid, and methylated to form a nitrile which, on hydrolysis, yielded an acid identical with the one, regarded as β-(4-benzoyl-3-methoxyphenoxy)propionic acid, which was isolated from the Friedel–Crafts reaction product. In addition, both β-(4-benzoyl-3-hydroxyphenoxy)propionic acid and its nitrile were decomposed by alkali to give 2 : 4-dihydroxybenzophenone, identified by direct comparison (mixed m. p.) with a specimen made by Komarowski and von Kostanecki's method (*Ber.*, **1894**, **27**, 1997). β-(2-Benzoyl-5-methoxyphenoxy)propionic acid gave no colour with ferric chloride and dissolved in aqueous sodium hydroxide to form a colourless solution, but when it was warmed or kept a yellow colour developed and acid then precipitated 2-hydroxy-4-methoxybenzophenone, the m. p. of which was not depressed by admixture with an authentic specimen. β-(4-Benzoyl-3-methoxyphenoxy)propionic acid, which also gave no colour with ferric chloride or sodium hydroxide, was decomposed by the latter reagent to give 4-hydroxy-2-methoxybenzophenone, which does not appear to have been previously described. It does not colour ferric chloride but it gives a very faintly yellow solution with sodium hydroxide.

Similar properties were observed with the veratroyl analogues, and they were converted into the expected benzophenones with alkali. 4-Hydroxy-2 : 3′ : 4′-trimethoxybenzophenone, obtained from β-(3-methoxy-4-veratroylphenoxy)propionic acid, melted at 179°, and gave no colour with ferric chloride and a pale yellow solution in sodium hydroxide. The substance to which Ray and his collaborators allotted this constitution melted at 175° and gave a faint ferric chloride reaction, yet there seems no doubt that these substances are identical. (The yellowing with alkali that we observed in this compound and the benzene analogue may be caused by traces of the *o*-hydroxy-isomer, but the absence of a ferric chloride reaction indicates that this is not present in appreciable quantity.)

Benzoyl chloride reacts with *m*-di-2′-carbomethoxyethoxybenzene in the presence of aluminium chloride to give 4-benzoyl-1 : 3-di-2′-carbomethoxyethoxybenzene (II; R = O·CH₂·CH₂·CO₂Me, R′ = CO₂Me, R″ = H). Acid hydrolyses this to the corresponding dicarboxylic acid, and treatment with sodium hydroxide at room temperature degrades this acid to β-(4-benzoyl-3-hydroxyphenoxy)propionic acid, identical with the substance described above. Further action of alkali gives 2 : 4-dihydroxybenzophenone. 4-Benzoyl-1 : 3-di-2′-cyanoethoxybenzene (II; R = O·CH₂·CH₂·CN, R′ = CN, R″ = H) has been obtained from benzoyl chloride and 1 : 3-di-2′-cyanoethoxybenzene.

All attempts to cyclise compounds of the type (II) with basic catalysts have so far been unsuccessful because they induce loss of the side chain so readily.

Reduction of 2-hydroxy-4-methoxybenzophenone by Clemmensen's method gave only a poor yield of 2-hydroxy-4-methoxydiphenylmethane, and so the possibility of condensing this substance with acrylonitrile and reoxidation to the ketone was not pursued in an attempt to prepare compounds of the type (II) that was made before the present work was done.

Experimental.

Reaction of Benzoyl Chloride with β-m-Methoxyphenoxypropionitrile.—A solution of aluminium chloride (28 g.) in nitrobenzene (80 c.c.) was slowly added, at room temperature, to a mixture containing *β-m*-methoxyphenoxypropionitrile (12·7 g.), benzoyl chloride (10 g.), and nitrobenzene (20 c.c.). After

being kept for 18 hours, the product was decomposed with ice, and nitrobenzene was distilled in steam. The residue was washed, first with dilute acid and then with water, and dissolved in ether. This solution was washed repeatedly with small volumes of aqueous sodium hydroxide (5%), and the aqueous layer was run, as quickly as possible, into dilute hydrochloric acid. A crystalline precipitate separated, and recrystallisation of this from alcohol afforded *β-(4-benzoyl-3-hydroxyphenoxy)propionitrile* as needles (4·5 g.), m. p. 109° raised to 110° by further recrystallisation from the same solvent (Found: C, 71·8; H, 4·5; N, 5·3. $C_{16}H_{13}O_3N$ requires C, 71·9; H, 4·9; N, 5·2%). The ethereal solution was then washed until free from alkali, dried (Na_2SO_4), and evaporated. The residue, which failed to crystallise, was hydrolysed by boiling it with concentrated hydrochloric acid for 7 hours, and then extracted with ether. From this solution, by extraction with sodium carbonate and subsequent acidification, extraction with ether, drying, and evaporation, a gummy product was obtained. This crystallised in contact with alcohol to give a solid (2·9 g.), m. p. 130—138°. Recrystallisation from alcohol afforded *β-(2-benzoyl-5-methoxyphenoxy)propionic acid*, as prisms, m. p. 138·5—140° (Found: C, 68·1; H, 5·3. $C_{17}H_{14}O_5$ requires C, 68·0; H, 5·3%). When this was heated on a steam-bath with sodium hydroxide solution (10%) for a few minutes, the originally colourless solution became deep yellow and on acidification yielded 2-hydroxy-4-methoxybenzophenone, m. p. 63° (after recrystallisation), identical (mixed m. p.) with an authentic specimen (Found: C, 73·8; H, 5·4. Calc. for $C_{14}H_{12}O_3$: C, 73·7; H, 5·3%).

β-(4-Benzoyl-3-hydroxyphenoxy)propionic Acid.—When the above nitrile was boiled with concentrated hydrochloric acid for 8—10 hours the corresponding *acid* was obtained; it crystallised from alcohol as needles, m. p. 173—175° sintering at 165° (Found: C, 66·9; H, 4·9. $C_{16}H_{14}O_5$ requires C, 67·1; H, 4·9%). This acid and the corresponding nitrile, when boiled for 5 minutes with aqueous sodium hydroxide (10%), gave a yellow solution from which acid precipitated 2 : 4-dihydroxybenzophenone, m. p. 143° (after recrystallisation), identical (mixed m. p.) with an authentic specimen.

β-(4-Benzoyl-3-methoxyphenoxy)propionic Acid.—β-(4-Benzoyl-3-hydroxyphenoxy)propionitrile (1 g.) in acetone (10 c.c.) was refluxed for 6 hours with methyl iodide (5 c.c.) and dry potassium carbonate (5 g.). After evaporation of the solvent, water was added and the precipitate was recrystallised from alcohol. *β-(4-Benzoyl-3-methoxyphenoxy)propionitrile* separated as needles (0·8 g.), m. p. 91° (Found: C, 72·5; H, 5·4. $C_{17}H_{15}O_3N$ requires C, 72·6; H, 5·3%). Hydrolysis of this nitrile was effected by boiling it with hydrochloric acid for 1½ hours. The product was extracted with sodium carbonate, as hydrolysis was incomplete, and acidification afforded a precipitate, m. p. about 70°. When this was rubbed with a little cold sodium hydroxide (10% solution) a colourless sodium salt crystallised, decomposition of which gave *β-(4-benzoyl-3-methoxyphenoxy)propionic acid*, which separated from aqueous methanol as prisms that fell to a crystalline powder, m. p. 83—84°, when dried *in vacuo* (Found: C, 68·0; H, 5·2. $C_{17}H_{16}O_5$ requires C, 68·0; H, 5·3%). [Acidification of the yellow alkaline solution from which the above sodium salt separated gave some β-(4-benzoyl-3-hydroxyphenoxy)propionic acid, and longer boiling during the hydrolysis caused considerable demethylation.] β-(4-Benzoyl-3-methoxyphenoxy)propionic acid and the corresponding nitrile were decomposed when warmed with aqueous sodium hydroxide (10%) to give *4-hydroxy-2-methoxybenzophenone*, which crystallised from alcohol as prisms, m. p. 124° (Found: C, 74·0; H, 5·2. $C_{14}H_{12}O_3$ requires C, 73·7; H, 5·3%).

Reaction of Benzoyl Chloride with Ethyl β-m-*Methoxyphenoxypropionate.*—Aluminium chloride (15·6 g.) in nitrobenzene (80 c.c.) and ethyl *β-m*-methoxyphenoxypropionate (8·8 g.) and benzoyl chloride (5·6 g.) in nitrobenzene (20 c.c.) were mixed and treated as above. The ethereal extract was then submitted to the following procedures.

(i) It was extracted with sodium carbonate (10%); acidification of the aqueous layer yielded material (0·7 g.) which crystallised from alcohol, giving β-(2-benzoyl-5-methoxyphenoxy)propionic acid, m. p. 135—137° raised by admixture with the acid, m. p. 138·5—140°, above.

(ii) It was repeatedly extracted with small quantities of aqueous sodium hydroxide (5%) which was run directly into hydrochloric acid; crystals (2·0 g.) separated, and on recrystallisation from alcohol afforded β-(4-benzoyl-3-hydroxyphenoxy)propionic acid, m. p. 173—175° not depressed by admixture with that prepared as above.

(iii) The ethereal layer was washed with water, dried ($CaCl_2$), and evaporated, yielding a yellow gum (9·5 g.); this failed to solidify and was hydrolysed by prolonged boiling with dilute hydrochloric acid. Non-acidic material was removed by partition between ether and sodium carbonate solution, and acid precipitated, from the aqueous layer, a substance that solidified. Careful fractional crystallisation from alcohol ultimately yielded β-(2-benzoyl-5-methoxyphenoxy)propionic acid (3—4 g.), m. p. 138—140° (from alcohol). From the mother-liquors there was obtained β-(4-benzoyl-3-methoxyphenoxy)propionic acid (0·4 g.), m. p. 83—85°. This had been purified by conversion into the sparingly soluble sodium salt and subsequent recrystallisation from aqueous methanol. Both specimens were identified (mixed m. p.) with the substances described above.

Reaction between Veratroyl Chloride and Ethyl β-m-*Methoxyphenoxypropionate.*—A mixture of veratroyl chloride (3·5 g.) and ethyl *β-m*-methoxyphenoxypropionate (4·4 g.) in nitrobenzene (20 c.c.) was treated, at room temperature, with aluminium chloride (7 g.) in nitrobenzene (25 c.c.) and submitted to the procedure described above. From the resulting product, rapid extraction with aqueous sodium hydroxide and acidification gave *β-(3-hydroxy-4-veratroylphenoxy)propionic acid*, which crystallised from alcohol and was obtained after repeated recrystallisation, as plates, m. p. 175—177° (0·5 g.) (Found: C, 62·2; H, 5·5. $C_{18}H_{18}O_7$ requires C, 62·4; H, 5·2%). The non-phenolic fraction failed to crystallise and was therefore hydrolysed by prolonged boiling with dilute hydrochloric acid. The resulting acids were extracted with sodium carbonate; fractional crystallisation from alcohol and aqueous alcohol afforded *β-(5-methoxy-2-veratroylphenoxy)propionic acid* (0·75 g.), which separated from alcohol as prisms, m. p. 135—136° (Found: C, 63·5; H, 5·7. $C_{19}H_{20}O_7$ requires C, 63·3; H, 5·6%). From the mother-liquors there was also obtained *β-(3-methoxy-4-veratroylphenoxy)propionic acid* (0·7 g.), which crystallised from aqueous alcohol as woolly needles, m. p. 127—129° (Found: C, 63·2; H, 5·6%). These three acids were all decomposed by being heated with aqueous sodium hydroxide solution: β-(3-hydroxy-

4-veratroylphenoxy)propionic acid gave 2 : 4-*dihydroxy*-3′ : 4′-*dimethoxybenzophenone*, which separated from alcohol as clear prisms, m. p. 149° (Found : C, 65·7; H, 5·4. $C_{15}H_{14}O_5$ requires C, 65·7; H, 5·1%); β-(5-methoxy-2-veratroylphenoxy)propionic acid gave 2-hydroxy-4 : 3′ : 4′-trimethoxybenzophenone, m. p. 141° not depressed by admixture with an authentic specimen, and β-(3-methoxy-4-veratroylphenoxy)propionic acid gave 4-hydroxy-2 : 3′ : 4′-trimethoxybenzphenone, which crystallised from alcohol as plates m. p. 179° (Found : C, 67·0; H, 5·7. Calc. for $C_{16}H_{16}O_5$: C, 66·7; H, 5·6%).

Reaction between Veratroyl Chloride and β-m-*Methoxyphenoxypropionitrile.*—Aluminium chloride (29 g.) in nitrobenzene (80 c.c.) was slowly added at room temperature to a mixture of veratroyl chloride (15 g.) and β-*m*-methoxyphenoxypropionitrile (13·2 g.) in nitrobenzene (20 c.c.). Next day, the mixture had set to a jelly and it was decomposed and steam-distilled, etc., as above. The product was dissolved in ether (1500 c.c.), and the solution was extracted with aqueous sodium hydroxide which was subsequently acidified and extracted with ethyl acetate. Both the phenolic fraction thus obtained (17 g.) and the non-phenolic part isolated from the ether (7·5 g.) failed to crystallise.

Non-phenolic fraction. (*a*) When it was boiled with concentrated hydrochloric acid for 5 hours it afforded some β-(5-methoxy-2-vertroylphenoxy)propionic acid, m. p. 133—135° (after two crystallisations from alcohol). (*b*) The non-phenolic substance (3·4 g.) was boiled with absolute alcohol (50 c.c.) in which sodium (0·23 g.) had been dissolved. After one hour addition of 2N-hydrochloric acid produced a precipitate, recrystallisation of which from alcohol gave 2-hydroxy-4 : 3′ : 4′-trimethoxybenzophenone (1·9 g.), m. p. 141°. Next day the aqueous solution had deposited small crystals (m. p. approx. 175°) (0·5 g.), and after two recrystallisations from alcohol this solid was shown to be 4-hydroxy-2 : 3′ : 4′-trimethoxybenzophenone, m. p. 179°. (Identifications were by mixed m. p. determination.)

Phenolic fraction. (*a*) When this substance was boiled with concentrated hydrochloric acid for 1½ hours it gave a product from which sodium carbonate extracted a little β-(3-hydroxy-4-veratroylphenoxy)propionic acid, m. p. 174—177° after two recrystallisations from alcohol. (*b*) This material was boiled with sodium hydroxide (10%) until no more ammonia was evolved (about 15 minutes), and acidification then yielded a gum that crystallised slowly in contact with alcohol. Recrystallisation from alcohol afforded 2 : 4-dihydroxy-3′ : 4′-dimethoxybenzophenone. (*c*) When this fraction was shaken with sodium hydroxide and methyl sulphate at room temperature, a good yield of 2 : 4 : 3′ : 4′-tetramethoxybenzophenone, m. p. 107°, separated. (*d*) The phenolic fraction (2 g.) was refluxed for 4 hours with methyl iodide (5 c.c.) and dry potassium carbonate (5 g.) in acetone (50 c.c.) and gave, after addition of water, a product that partly crystallised : the crystals were 2-hydroxy-4 : 3′ : 4′-trimethoxybenzophenone (*i.e.*, methylation was incomplete). The neutral residue, obtained after treatment with alkali, was then boiled with sodium hydroxide (10%) for 15 minutes, and the insoluble portion solidified and was removed. It was 2 : 4 : 3′ : 4′-tetramethoxybenzophenone. Acidification of the aqueous layer then afforded a solid from which 4-hydroxy-2 : 3′ : 4′-trimethoxybenzophenone, m. p. 179°, was obtained. (*e*) When the neutral fraction produced in (*d*) was hydrolysed with concentrated hydrochloric acid and the resulting product was dissolved in ether and extracted with aqueous sodium carbonate, a small quantity of β-(3-methoxy-4-veratroylphenoxy)propionic acid, m. p. 127—129°, was obtained. (Identification of all the above substances was by mixed m. p. determination.)

m-*Di-2′-carboxyethoxybenzene.*—*m*-Di-2′-cyanoethoxybenzene (10 g.) (made by Cook and Reed's method, *J.*, 1945, 920), sodium being used as catalyst but the reaction being carried out under reflux conditions) was boiled with concentrated hydrochloric acid (100 c.c.). After about 30 minutes the nitrile had dissolved and the acid crystallised rapidly. Recrystallisation from alcohol gave m-*di-2′-carboxyethoxybenzene* as needles, m. p. 184°, sintering at 175°. The m. p. was not changed by further recrystallisation (Found : C, 56·6; H, 5·6. $C_{12}H_{14}O_6$ requires C, 56·7; H, 5·5%) (yield 11·3 g.). This acid (13 g.) was esterified by 2 hours' boiling with methyl alcohol (130 c.c.) and sulphuric acid (5 c.c.) and afforded m-*di-2′-carbomethoxyethoxybenzene* as needles (11 g.), m. p. 72·5—74·5° after two recrystallisations from methyl alcohol (Found : C, 59·3; H, 6·4. $C_{14}H_{18}O_6$ requires C, 59·6; H, 6·4%). The ethyl ester, prepared in a similar way, was obtained as needles, m. p. 39—41°.

4-*Benzoyl*-1 : 3-*di-2′-carbomethoxyethoxybenzene.*—Aluminium chloride (53 g.) in nitrobenzene (200 c.c.) was added to a mixture of *m*-di-2′-carbomethoxyethoxybenzene (28 g.) and benzoyl chloride (14 g.) in nitrobenzene (100 c.c.) at 0°. After 12 hours the mixture was worked up in the usual way; the product crystallised from alcohol as orange prisms. Further recrystallisation from the same solvent gave 4-*benzoyl*-1 : 3-*di-2′-carbomethoxyethoxybenzene* as large, almost colourless prisms, m. p. 83—85° (14 g.) (Found : C, 65·1; H, 5·8. $C_{21}H_{22}O_7$ requires C, 65·3; H, 5·7%). Treatment with aqueous-alcoholic sodium hydroxide (10%) at room temperature for 2 hours afforded β-(4-benzoyl-3-hydroxyphenoxy)propionic acid, m. p. 173—175°, identical (mixed m. p.) with the compound prepared as above, and 1 hour's heating with absolute alcohol containing an equivalent of sodium ethoxide gave a high yield of 2 : 4-dihydroxybenzophenone. Hydrolysis to 4-*benzoyl*-1 : 3-*di-2′-carboxyethoxybenzene* was effected by prolonged boiling of it with 2N-hydrochloric acid. This acid crystallised from water or alcohol as prisms, m. p. 159°, sintering at 151° (Found : C, 64·2; H, 5·0. $C_{19}H_{18}O_7$ requires C, 63·7; H, 5·0%).

4-*Benzoyl*-1 : 3-*di-2′-cyanoethoxybenzene.*—This *compound* was prepared from *m*-di-2′-cyanoethoxybenzene and benzoyl chloride in the usual way. It crystallised from alcohol as needles, m. p. 83—85° (Found : C, 70·8; H, 5·2. $C_{19}H_{16}O_5N_2$ requires C, 71·2; H, 5·0%).

2-*Hydroxy-4-methoxydiphenylmethane.*—2-Hydroxy-4-methoxybenzophenone (15 g.) was refluxed with amalgamated zinc (150 g.), concentrated hydrochloric acid (180 c.c.), and water (120 c.c.) for 24 hours, further 50-c.c. portions of acid being added from time to time to maintain evolution of hydrogen. The product was then extracted with ether, and the yellow gum that remained after drying and evaporation of the solvent partly crystallised. The solid was separated by prolonged suction, and recrystallisation from light petroleum (b. p. 60—80°) afforded 2-*hydroxy-4-methoxydiphenylmethane* as needles, m. p. 51—53° (Found : C, 78·5; H, 6·6. $C_{14}H_{14}O_2$ requires C, 78·5; H, 6·5%).

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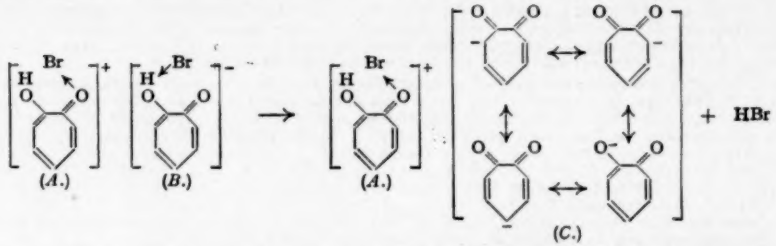
496. *Tropolones. Part III.* Halogenated Derivatives of Tropolone.*

By J. W. Cook, A. R. M. Gibb, and R. A. Raphael.

The courses of the bromination and iodination of tropolone are described and interpreted. The constitutions tentatively formulated in Part I (*J.*, 1951, 503) for the brominated tropolones have been confirmed by conversion of these derivatives into benzenoid compounds of known orientation.

As part of a detailed examination of the reactions of tropolone its bromination and iodination have been studied. Treatment of the compound in cold carbon tetrachloride or glacial acetic acid with a half-molecular proportion of bromine resulted in the immediate precipitation of a highly insoluble scarlet complex without the observable evolution of hydrogen bromide. The yield of this product was essentially the same when one and two molecular proportions of bromine were used, but was approximately halved when only a quarter-molecular proportion was employed. The complex was readily decomposed by water or methanol but could be crystallised unchanged from dry acetic acid; it remained unaltered for several months in a completely dry atmosphere. Analysis of the complex indicated that it contained one atom of bromine for each tropolone nucleus, a result confirming the stoicheiometry of the reaction. On being heated to 110° the complex decomposed to a colourless crystalline mass with concomitant evolution of hydrogen bromide; quantitative determination of the latter indicated that one molecule was formed from one molecule of the complex [on the assumption that this is $(C_7H_6O_2)_2,Br_2$]. Fractional sublimation of the colourless solid product gave tropolone, tropolone hydrobromide, and a mixture of brominated tropolones from which mono- and dibromotropolones could be isolated. Decomposition of the complex with water, sodium hydroxide solution, or methanol gave essentially similar products except that no tropolone hydrobromide was encountered.



To explain these phenomena the above ionic formulation (*A*–*B*) for the complex is proposed. The cation (*A*) is regarded as being formed by the donation of the carbonyl-oxygen lone pair to the unoccupied 4*p* orbital of the bromonium cation produced by heterolytic fission of the bromine molecule, thus yielding a structure analogous to the oxonium salts. The anion (*B*) is conceived to be the result of hydrogen bonding between the hydroxylic hydrogen atom of tropolone and the bromine anion, a construction closely resembling that of the well-established hydrofluoride ion ($F^-H\leftarrow F^-$). The formation of such a complex may thus be seen as a further manifestation of the amphoteric nature of the tropolone ring system.

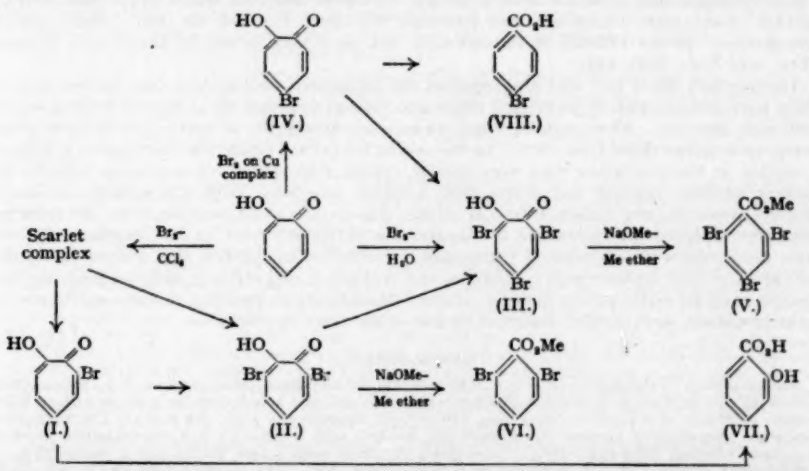
It is reasonable to assume that thermal activation of the complex results in the ejection of one molecule of hydrogen bromide from the anion (*B*) to form the mesomeric anion (*C*). The cation (*A*) now functions as a source of the well-established brominating agent, bromonium cation, which interacts with the mesomeric anion (*C*) to produce brominated tropolones at the indicated points of electron access, tropolone itself being thereby regenerated from cation (*A*). Furthermore, the driving force of the reaction with hydroxylic solvents at room temperature may be taken to be the ease of formation of hydroxonium ion by interaction of anion (*B*) with a water molecule.

It is seen that this structural concept accommodates both qualitatively and quantitatively the above-mentioned experimental results concerning the formation, properties, and dis-

* Part II, *J.*, 1951, 695.

proportionation of the complex. The striking scarlet colour of the bromine complex is also in harmony with this formulation, being almost certainly due to that " type of absorption characterised by very great intensity, shown by certain crystals containing adjacent ions in different valency states " (Bowen, " Chemical Aspects of Light," Oxford Univ. Press, 1946, p. 144). Prussian-blue is the best-known example of this phenomenon. On this view, the intense long-wave absorption of the tropolone–bromine complex may be attributed to a ready anion–cation electron interchange resulting in an interconversion of their functions. That the colour is a crystal-structure effect is borne out by the fact that the acetic acid solution of the complex is pale yellow; this phenomenon is exactly paralleled in the case of $K_4Mn(CN)_6$, the dark blue crystals of which give a pale yellow solution (Bowen, *loc. cit.*). It is interesting that similar formation of highly coloured complexes with halogens has been observed in the case of a number of polycyclic aromatic compounds (for review see Cook, *Ann. Reports*, 1942, **39**, 167), and the recorded analyses of some of the compounds indicate a structure comparable with that of the above tropolone complex. Recently, evidence has accumulated that benzene itself forms complexes with halogens in solution, the aromatic nucleus functioning as an electron donor (for review and references see Keefer and Andrews, *J. Amer. Chem. Soc.*, 1950, **72**, 5170).

The mono- and di-bromotropolones obtained from the decomposition of the complex were found to be identical with the corresponding derivatives obtained by bromination of *cyclo*heptane-1 : 2-dione. Bromination of the monobromotropolone in acetic acid with one mole of bromine gave the same dibromotropolone. Further bromination of the dibromotropolone in acetic acid needed the application of heat and a tribromotropolone was obtained identical with that prepared by the exhaustive bromination of *cyclo*heptane-1 : 2-dione (Part I). The same tribromotropolone was also produced directly from tropolone by bromination in aqueous solution. In an attempt to avoid the aforementioned complications arising from the presence of the hydroxy-carbonyl system of tropolone, copper tropolone was brominated, as the formation of a chelate salt would be expected to annul the amphoteric nature of the parent compound. From the mixture of brominated tropolones thus obtained there was readily isolated in fair yield a new, higher-melting monobromotropolone (m. p. 188°). A small amount of this substance has also been isolated from the mother-liquors of the bromination of *cyclo*heptane-1 : 2-dione. Further bromination of this new product gave the same tribromotropolone encountered above. The formation and interrelationship of these bromo-derivatives may be formulated as in the annexed scheme.



The orientation of the derivatives was carried out in the following manner. The methyl ether of tribromotropolone on being heated for 30 minutes with methanolic sodium methoxide underwent smooth conversion into methyl 2 : 4 : 6-tribromobenzoate (V) identified by comparison with an authentic sample; this confirms the $\alpha\alpha'\gamma$-structure (III) for the parent com-

pound. On similar treatment the methyl ether of dibromotropolone rearranged to methyl 2 : 6-dibromobenzoate (VI); this not only establishes the correctness of the αα′-structure (II) but also indicates that the lower-melting monobromotropolone from which (II) may be derived must be the α-compound (I). An attempt to confirm the latter assignment by rearrangement of α-monobromotropolone methyl ether was abortive; much ionic bromine was produced and a minute yield of an impure bromine-free tropolone-like material was obtained. Potassium hydroxide fusion of (I), however, gave a small yield of salicylic acid (B. J. Abadir, personal communication). These assignments indicated that the higher-melting monobromotropolone should be the γ-isomer (IV); this structure was confirmed by the sodium methoxide rearrangement of its methyl ether to methyl *p*-bromobenzoate, characterised by hydrolysis to the free acid (VIII). It is seen that these results confirm Dewar's predictions (*Nature*, 1950, **166**, 790) that the 3 (α), 5 (γ) and 7 (α′) positions of tropolone would be those most liable to electrophilic attack. They do not, with regard to bromination at least, support the author's contention that the γ-position should be more prone to such substitution than the α- and the α′-position.

Theoretically an alternative disruption of the tropolone–bromine complex (*A–B*) may be visualised, namely, direct recombination to regenerate tropolone and bromine. This does not appear to occur to any appreciable extent in the case of the bromine complex, but seems to be the major reaction in the case of the tropolone–iodine complex which was prepared by the action of aqueous iodine–potassium iodide on tropolone. This black, crystalline complex was unaffected by water but was intrinsically much less stable than the corresponding bromine complex, iodine being slowly evolved at room temperature. This process was hastened by sublimation whereupon the complex broke up into iodine and tropolone.

The action of iodine in alkaline solution (*i.e.*, hypoiodite) on tropolone was found to follow an entirely different course. On the reactants being mixed in the cold a yellow crystalline precipitate was immediately formed. This, after purification, was identified as iodoform. The crude material obtained in an earlier experiment gave tropolone colour reactions, and this was responsible for the mistaken suggestion (Part I; *loc. cit.*) that it was an iodotropolone. Acidification of the alkaline filtrate gave a product from which 2 : 4 : 6-tri-iodophenol could be readily isolated, in conformity with the recently reported finding by Doering and Knox (*J. Amer. Chem. Soc.*, 1951, **73**, 828), who do not seem to have observed the formation of iodoform. The yield of iodoform varied with the reaction conditions, and it is probably a secondary product rather than a product inherent in the rearrangement which leads to the iodinated phenol. It may be noted that stipitatic acid, a natural tropolone, has been found to give an iodoform reaction (Birkinshaw, Chambers, and Raistrick, *Biochem. J.*, 1942, **36**, 250). Many similar "exceptions" to the validity of the iodoform test have been noted by Booth and Saunders (*Chem. and Ind.*, 1950, 824).

Doering and Knox (*loc. cit.*) also reported the conversion of tropolone into benzoic acid by fusion with potash, and of its methyl ether into methyl benzoate by means of boiling sodium methoxide solution. These results, which we have confirmed, are at variance with those which we reported earlier (Part I, *loc. cit.*). In the case of the potash fusion the discrepancy is difficult to explain as the conditions were very similar, except that we used a hard-glass tube for the reaction whereas Doering and Knox used a nickel crucible. With the sodium methoxide reaction, however, our earlier failure is clearly due to the short reaction time (30 minutes) which we employed, in contrast to the longer time (12 hours) used by the American workers. These conversions into benzenoid compounds by alkaline hypoiodite and sodium methoxide have already been realised with colchiceine and colchicine, respectively, and the evidence that tropolone and its methyl ether undergo similar transformations provides valuable corroboration of the tropolone methyl ether structure of one of the rings of colchicine.

Experimental.

Bromination of Tropolone.—(*a*) To a cold solution of tropolone (56·1 mg.) in dry carbon tetrachloride (3 c.c.) contained in a weighed filter beaker was added a solution of bromine in carbon tetrachloride (0·34 c.c. of a solution containing 110 mg. of bromine per c.c.; 0·5 mol.). The orange-red precipitate immediately formed was filtered off, washed with a little carbon tetrachloride, dried *in vacuo*, and weighed (68·4 mg., 74%). The yields obtained with 1 mol. (85%) and 2 mols. (81%) of bromine were similar, but that produced by 0·25 mol. was 31%. The complex dissolved in hot glacial acetic acid to give a yellow solution from which it crystallised on cooling as a mass of scarlet needles; accurate analysis was difficult owing to its ready reaction with atmospheric moisture [Found: C, 39·9; H, 3·5; Br, 40·3. $(C_7H_4O_2)_3.Br_2$ requires C, 41·6; H, 3·0; Br, 39·6%]. On being heated to 110° the substance sintered and rapidly decolorised to form a white, crystalline mass which melted unsharply to a clear liquid at 152—163°. On careful heating at 70° (bath-temp.)/10^{-5} mm. the complex slowly

sublimed with only slight decomposition. A sample of the complex (84·2 mg.) was heated at 140° for 45 minutes in a slow stream of nitrogen, the issuing gases being bubbled through sodium hydroxide solution (5 c.c. of N.). The latter was treated with excess of nitric acid and silver nitrate, and the precipitated silver bromide was coagulated by boiling, filtered off, washed, dried, and weighed [Found: AgBr, 32·3 mg. Calc. for the production of one mol. of HBr from $(C_7H_4O_2)_2.Br_2$: AgBr, 39·2 mg.].

The complex (127 mg.) was heated in an oil-bath at 110° at atmospheric pressure in a sublimation apparatus until decolourisation was complete. The bath was then cooled to 60° and the apparatus evacuated to 20 mm.; the crystalline sublimate thus obtained (6 mg.) had m. p. 48—50° undepressed on admixture with tropolone. The bath-temperature was then raised to 120° and the pale yellow sublimate formed (42 mg.), m. p. 83—94°, was purified by repeated crystallisation from *cyclo*hexane to yield pure α-monobromotropolone (17 mg.), m. p. 105—107°, undepressed on admixture with an authentic specimen; from the number of crystallisations required to attain purity it is probable that the product contained a little of the higher-melting γ-monobromotropolone (see below). The residue remaining in the sublimation apparatus was dissolved in a small quantity of warm glacial acetic acid; on being cooled the solution deposited small rosettes of needles (11 mg.), m. p. 154—157° undepressed on admixture with dibromotropolone (m. p. 157—158°). Evaporation of the acetic acid mother-liquors to dryness under reduced pressure left a white crystalline mass (38 mg.), insoluble in hydrocarbon solvents but crystallising from a small volume of methanol or ethyl methyl ketone in long needles, m. p. 130—132° (Kofler block), resolidifying slowly on further heating to a mass of needles which did not melt below 250°. This compound was found to be *tropolone hydrobromide* identical with an authentic sample prepared by passing hydrogen bromide into a benzene solution of tropolone (Found: Br, 38·6. $C_7H_7O_2Br$ requires Br, 39·35%). On being heated under reflux with a small volume of ethyl acetate the hydrobromide slowly dissolved; evaporation to dryness gave tropolone (18 mg.), crystallising from light petroleum (b. p. 40—60°) in needles, m. p. 49—50° undepressed on admixture with an authentic specimen.

(*b*) To a cold solution of tropolone (57 mg.) in water (5 c.c.) was rapidly added a solution of bromine (290 mg., 3·5 mols.) in potassium bromide solution. The yellow precipitate formed was filtered off, washed with water, and crystallised from methanol, from which solvent it formed yellow needles (116 mg.), m. p. 123° undepressed on admixture with the tribromotropolone obtained as described in Part I. The action of ethereal diazomethane gave *tribromotropolone methyl ether*, crystallising from a small volume of light petroleum (b. p. 40—60°) in yellow nodules, m. p. 123—124° (strongly depressed on admixture with starting material) (Found: C, 26·05; H, 1·5. $C_8H_5O_2Br_3$ requires C, 25·8; H, 1·35%).

Bromination of α-Monobromotropolone (I).—To a cold solution of α-monobromotropolone (200 mg.) in acetic acid (3 c.c.) was added an acetic acid solution of bromine (160 mg., 1 mol.). After a few minutes a yellow crystalline precipitate (210 mg.) separated. This was filtered off; evaporation of the filtrate to dryness gave dibromotropolone (60 mg.), m. p. 156—157° after one crystallisation from methanol.

The precipitate obtained melted at 80—85° with evolution of hydrogen bromide, rapidly resolidifying, and remelting at 145—153°. It rapidly dissolved in a small volume of warm methanol; on being cooled the solution deposited needles of pure dibromotropolone, m. p. 157—158° (118 mg.). The low-melting intermediate was found to be identical with dibromotropolone hydrobromide prepared by treating the parent compound with an acetic acid solution of hydrogen bromide.

Mixed-melting-point determination showed that the dibromotropolone prepared as above was identical with that obtained as described in Part I.

Bromination of Dibromotropolone (II).—A solution of dibromotropolone (47 mg.) in acetic acid (2 c.c.) was treated with an acetic acid solution of bromine (40 mg., 1·5 mols.). No action was apparent in the cold, but the bromine colour was slowly discharged when the reaction mixture was heated under gentle reflux for 30 minutes. Evaporation to dryness under reduced pressure and crystallisation from methanol gave tribromotropolone (42 mg.), m. p. 123° undepressed on admixture with an authentic specimen.

Bromination of Copper Tropolone.—To a cold solution of copper tropolone (176 mg.) in chloroform (20 c.c.) was rapidly added a solution of bromine in chloroform (1·51 c.c. of a solution containing 135 mg. of bromine per c.c.; 2 mols.); a black, powdery precipitate was formed. After 16 hours at room temperature an equal volume of water was added and the reaction mixture treated with hydrogen sulphide. Extraction with ether, followed by drying (Na_2SO_4) and evaporation, yielded a dark oil which rapidly solidified; sublimation of the product at 110—120°/0·5 mm. gave a pale yellow solid (196 mg.) melting over the range 85—110°. Fractional crystallisation from light petroleum (b. p. 60—80°) gave two main fractions, m. p. 96—103° (28 mg.) and m. p. 176—180° (46 mg.) respectively. Two crystallisations of the latter from a small quantity of ethanol gave pure *γ-monobromotropolone* (22 mg.) as needles, m. p. 188° with vigorous sublimation (Found: C, 41·9; H, 2·7. $C_7H_5O_2Br$ requires C, 41·8; H, 2·5%). The lower-melting fraction, whose melting range was not markedly improved by further crystallisation, consisted of slightly impure α-monobromotropolone. The action of ethereal diazomethane on γ-monobromotropolone gave the *methyl ether* crystallising from light petroleum (b. p. 60—80°) in needles, m. p. 135—137° (Found: C, 44·9; H, 3·3. $C_8H_7O_2Br$ requires C, 44·7; H, 3·3%); a marked change in crystal form occurred before melting.

γ-Monobromotropolone (160 mg.) was also obtained by subjecting the crude fractions (2 g.; m. p. 68—80°) obtained from the bromination of *cyclo*heptane-1 : 2-dione to fractional crystallisation from ethanol.

Heating γ-monobromotropolone in acetic acid with excess of bromine (2·5 mols.) gave an almost quantitative yield of tribromotropolone, m. p. and mixed m. p. 123°.

Rearrangement of Brominated Tropolone Methyl Ethers.—(*a*) Tribromotropolone methyl ether (60 mg.) was added to a solution of sodium (50 mg.) in dry methanol (5 c.c.), and the reaction mixture heated under reflux for 30 minutes. The solvent was removed under reduced pressure, and water and

ether were added to the residue. Evaporation of the dried (Na_2SO_4) ethereal layer furnished an oil which rapidly solidified; crystallisation of the product from aqueous ethanol gave methyl 2 : 4 : 6-tribromobenzoate (26 mg.) as plates, m. p. 69—70° undepressed on admixture with an authentic specimen (m. p. 69—70°).

(*b*) Dibromotropolone methyl ether (46 mg.) was treated in a manner exactly analogous to (*a*). The product (32 mg.) crystallised from aqueous ethanol in platelets, m. p. 61—62° undepressed on admixture with authentic methyl 2 : 6-dibromobenzoate (m. p. 61—62°) (Found : C, 32·6; H, 2·3. Calc. for $C_8H_6O_2Br_2$: C, 32·7; H, 2·05%). The ester was prepared by the action of ethereal diazomethane on 2 : 6-dibromobenzoic acid obtained by Olivier's method (*Rec. Trav. chim.*, 1924, **43**, 872). On the two previous occasions that this ester has been mentioned in the literature the m. p. has been recorded as 83° (Buning, *Rec. Trav. Chim.*, 1921, **40**, 350) and 78° (Sudborough, *J.*, 1895, **67**, 595); in these cases the compound was prepared by reaction of the acid chloride with methanol or sodium methoxide. Dimorphism seems to be the most likely explanation of this discrepancy; the m. p. of our ester was not changed by repeated crystallisation from various solvents or by melting and resolidification.

(*c*) γ-Monobromotropolone methyl ether (108 mg.) was treated as in (*a*) with a solution of sodium (100 mg.) in methanol (5 c.c.). The residue obtained after removal of the solvent was treated with sodium hydroxide solution (5 c.c. of 2N.), and the mixture heated under reflux for 1 hour. The cooled solution was extracted once with ether to remove neutral impurities, and the aqueous layer acidified with dilute sulphuric acid. Extraction with ether, drying (Na_2SO_4), and evaporation furnished a brown solid which was purified by sublimation (140°/0·5 mm.). Crystallisation of the sublimate from benzene gave *p*-bromobenzoic acid (33 mg.) as tiny needles, m. p. 245—247° with rapid sublimation, undepressed on admixture with an authentic sample (m. p. 248—250°).

Iodination of Tropolone.—(*a*) To a solution of tropolone (100 mg.) in water (15 c.c.) was added a solution of iodine (150 mg.) in potassium iodide solution (5 c.c. of 10%). The black precipitate immediately formed (160 mg.) was filtered off, dried on porous tile, and crystallised from chloroform from which solvent it formed black lustrous needles decomposing at *ca.* 140°. Accurate analysis was difficult as the *complex* slowly evolved iodine even at room temperature [Found : I, 49·5. $(C_7H_6O_2)_2.I_2$ requires I, 50·9%]. When a solution of the complex in chloroform was shaken with sodium thiosulphate solution the colour was immediately discharged and evaporation of the colourless chloroform layer furnished tropolone. Attempted sublimation of the complex in a high vacuum resulted in complete dissociation into iodine and tropolone.

(*b*) To a cold solution of tropolone (200 mg.) in N-sodium hydroxide (30 c.c.) was rapidly added a solution of iodine (3 g.) in potassium iodide solution. The yellow crystalline precipitate immediately formed (140 mg.) was filtered off and crystallised from ethanol to give yellow plates, m. p. 119—120° undepressed on admixture with iodoform (m. p. 119—120°). The alkaline filtrate was acidified with dilute sulphuric acid, the iodine colour discharged with a little sodium hydrogen sulphite solution, and the resulting solid filtered off. Crystallisation of this product from acetic acid gave needles (260 mg.), m. p. 153—155° undepressed on admixture with 2 : 4 : 6-tri-iodophenol (m. p. 155—156°).

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THE UNIVERSITY, GLASGOW, W.2. [*Received, May 2nd*, 1951.]

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497. *Melanin and its Precursors. Part III.* New Syntheses of 5 : 6-Dihydroxyindole and its Derivatives.*

By JOHN D. BU'LOCK and JOHN HARLEY-MASON.

As previously reported briefly (*Nature*, 1950, **166**, 1036), β-3 : 4-dihydroxyphenyl-*N*-methylalanine and 2-3′ : 4′-dihydroxyphenylethylmethylamine have been converted directly into 5 : 6-dihydroxy-1-methylindole by oxidation with potassium ferricyanide and isomerisation of its resulting quinones with zinc acetate. Similar treatment of β-3 : 4-dihydroxyphenylalanine leads to the formation of 5 : 6-dihydroxyindole.

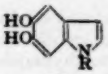
Oxidation of β-3 : 4-dihydroxyphenylalanine ethyl ester with potassium iodate gives a red crystalline iodo-quinone, and a similar iodo-quinone is obtained from 2-3′ : 4′-dihydroxyphenylethylmethylamine. Both products are isomerised by zinc acetate.

The mechanism of the zinc-catalysed isomerisation is discussed.

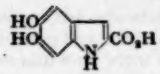
THE formation of the melanin intermediates 5 : 6-dihydroxyindole (I; R = H) and its 2-carboxylic acid (II) by enzymic oxidation of tyrosine has been established (Raper, *Biochem. J.*, 1927, **21**, 89; Dulière and Raper, *ibid.*, 1930, **24**, 239); the products were isolated as their *O*-methyl derivatives, which have not been successfully demethylated. (I) and (II) have since been synthesised by another and longer route (Beer, McGrath, Robertson, and Woodier, *J.*, 1949, 2061).

* Part II, *J.*, 1951, 703.

It appeared probable that these compounds should be obtainable from the primary oxidation product of tyrosine, 3 : 4-dihydroxyphenylalanine, by oxidation to the red quinone * (III)



(I.)



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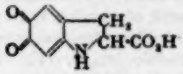
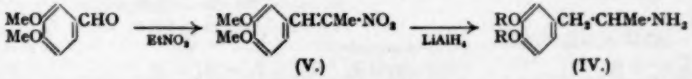


(III.)

followed by isomerisation. Oxidation by means of silver oxide had already been examined by Dulière and Raper (*loc. cit.*) who isolated the two *O*-methyl derivatives mentioned above. We have now found that oxidation is best effected by potassium ferricyanide, and that the quinone thus obtained is isomerised and simultaneously decarboxylated to give (I; R = H) by the action of zinc acetate. This use of zinc acetate was prompted by Fischer, Dérouaux, Lambot, and Lecomte's observation (*Bull. Soc. chim. Belg.*, 1950, **59**, 72) that adrenochrome is converted by this reagent into 3 : 5 : 6-trihydroxy-1-methylindole. This catalysed isomerisation of 2 : 3-dihydroindole-5 : 6-quinones is, in fact, general and of considerable preparative value : its mechanism is discussed below. The yields of (I; R = H) obtained by this process were, however, variable and rather low, apparently owing to oxidation or polymerisation of the product in the dilute solutions used. Much better yields were obtained by application of a similar procedure to β-3 : 4-dihydroxyphenyl-*N*-methylalanine, leading to the formation of 5 : 6-dihydroxy-1-methylindole (I; R = Me). Similar treatment of 2-3′ : 4′-dihydroxyphenylethylmethylamine (" epinine ") also gave (I; R = Me) in good yield.

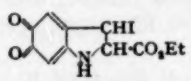
Oxidation of 2-3′ : 4′-dihydroxyphenylethylamine and 2-3′ : 4′-dihydroxyphenyl*iso*propylamine (IV; R = H) was next examined in the hope of obtaining 5 : 6-dihydroxyindole and its 2-methyl derivative. In both cases, however, only very small amounts of the indoles were formed, and the method was of no preparative value. (IV; R = H) was first obtained by Mannich and Jacobsohn (*Ber.*, 1910, **43**, 194). We adopted a more convenient synthesis in which veratraldehyde was condensed with nitroethane to give 1-(3 : 4-dimethoxyphenyl)-2-nitropropene (V), which was then reduced with lithium aluminium hydride to the amine (IV; R = Me). Demethylation with hydrobromic acid then gave the dihydroxy-amine (IV; R = H).

Although its structure is hardly in doubt, the quinone (III) has in fact never been isolated, since it is very soluble in water and the solutions decompose on concentration, and moreover it is not extracted from water by organic solvents. It seemed probable that a derivative of (III) containing a heavy substituent might be less soluble and thus more readily isolated. By the action of potassium iodate on β-3 : 4-dihydroxyphenyl-L-alanine ethyl ester we have obtained crystalline, sparingly soluble ethyl 2 : 3-dihydro-3-iodoindole-5 : 6-quinone-2-carboxylate (VI). On treatment with zinc acetate or alkali, isomerisation to ethyl 5 : 6-dihydroxy-

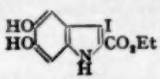


(V.) (IV.)

3-iodoindole-2-carboxylate (VII) occurred readily, indicating that the iodine atom did not occupy the 2-position in the quinone. This isomerisation also took place on storage of the solid material; the deep red crystals slowly became brown and recrystallisation then gave (VII). Reduction of (VI) gave a colourless leuco-compound which readily re-oxidised to the



(VI.)



(VII.)

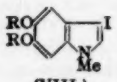
quinone. The preparation of (VI) starting from the racemic rather than the L-ester was much less satisfactory. The entrance of the iodine atom creates a new asymmetric centre, so that from the L-ester two diastereoisomers could be formed. The material isolated in this case appeared to be homogeneous, so that it is probable that in fact an asymmetric synthesis under

* This compound is frequently referred to as " hallachrome " on the basis of its supposed identity with the red pigment isolated from the polychæte worm *Halla parthenopoea* (Mazza and Stolfi, *Arch. Sci. biol.*, 1931, **16**, 182). This identity has now been disproved (Bu'Lock, Harley-Mason, and Mason, *Biochem. J.*, 1950, **47**, xxxii) and the alternative trivial name " dopachrome " is proposed for (III).

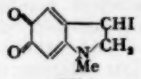
the influence of the neighbouring asymmetric atom takes place in the iodination, leading to a single product. In the case of the racemic ester, two products must necessarily be formed and this would make crystallisation more difficult.

Treatment of epinine with potassium iodate also led to the formation of a crystalline iodoquinone, which on treatment with acetic anhydride and pyridine gave a colourless diacetoxyiodo-1-methylindole, m. p. 145—146°, differing from 5 : 6-diacetoxy-2-iodo-1-methylindole, m. p. 153—155° (Bergel and Morrison, *J.*, 1943, 48). Our product was therefore the corresponding 3-iodo-compound (VIII; R = Ac), and the iodo-quinone was 2 : 3-dihydro-3-iodo-1-methylindole-5 : 6-quinone (IX). The action of zinc acetate on (IX) gave 5 : 6-dihydroxy-3-iodo-1-methylindole (VIII; R = H).

The results obtained from the oxidation of ten 2-3' : 4'-dihydroxyphenylethylamine derivatives, including four from our earlier work, indicate the scope of the reaction and are collected in the Table. Inspection reveals that, in general, better results were obtained with secondary amines; with the primary amines examined the failures or poor yields appeared



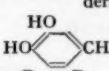
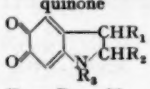
(VIII.)



(IX.)

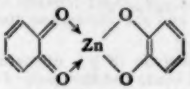
in most cases to be due to unsatisfactory cyclisation in the formation of the dihydroindole-quinone. This may be correlated with the basicity of the nitrogen atom which will be higher in the secondary amines, thus facilitating the cyclisation which involves anionoid attack on the *o*-benzoquinone nucleus formed as the first stage in the oxidation (cf. Robinson and Sugasawa, *J.*, 1932, 789; Bu'Lock and Harley-Mason, *J.*, 1951, 712).

The action of zinc ions in catalysing the rearrangement of dihydroindole-quinones is apparently unique, and no analogies could be found : its mechanism was therefore of particular interest. Oxidation of catechols in aqueous solution in the presence of zinc acetate led to the formation of insoluble, dark green complexes derived from two molecules of the catechol and one atom of zinc. These complexes are decomposed by acids and are clearly similar to those obtained by Criegee and Klonk (*Annalen*, 1949, **564**, 1) by the oxidation of " ene-diol " compounds in the presence of alkaline-earth metal hydroxides, and those obtained by Michaelis and Granick (*J. Amer. Chem. Soc.*, 1948, **70**, 624) by the alkaline reduction of 9 : 10-phenanthraquinone in the presence of the same metal ions. They are therefore formulated as in (X); as shown, one ring is benzenoid and the other *o*-quinonoid, although in fact each ring is at an

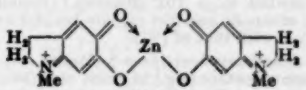
β-3 : 4-Dihydroxyphenylethylamine derivative 	Oxidising agent.	2 : 3-Dihydroindole-5 : 6-quinone 	Yield (quinone or isomerisation product).
$R_1 = R_2 = R_3 = H$ (Hydroxytyramine)	Ferricyanide	$R_1 = R_2 = R_3 = H$	Trace
	Iodate		Nil
$R_1 = R_3 = H$; $R_2 = Me$	Ferricyanide	$R_1 = R_3 = H$; $R_2 = Me$	Trace
	Iodate		Nil
$R_1 = R_2 = H$; $R_3 = Me$ (Epinine)	Ferricyanide	$R_1 = R_2 = H$; $R_3 = Me$	60%
	Iodate	$R_1 = I$, $R_2 = H$, $R_3 = Me$	50%
$R_1 = R_3 = H$, $R_2 = CO_2H$ (β-3 : 4-Dihydroxyphenylalanine)	Ferricyanide	$R_1 = R_3 = H$, $R_2 = CO_2H$	5—30%
	Iodate		Nil
$R_1 = R_3 = H$, $R_2 = CO_2Et$ (β-3 : 4-Dihydroxyphenylalanine ethyl ester)	Iodate	$R_1 = I$, $R_2 = CO_2Et$, $R_3 = H$	30%
$R_1 = H$, $R_2 = CO_2H$, $R_3 = Me$ β-(3 : 4-Dihydroxyphenyl-*N*-methylalanine)	Ferricyanide	$R_1 = H$, $R_2 = CO_2H$, $R_3 = Me$	40%
$R_1 = OH$, $R_2 = R_3 = H$ (Noradrenaline)	Ferricyanide	$R_1 = OH$, $R_2 = R_3 = H$	Nil
	Iodate	$R_1 = OH$, $R_2 = I$, $R_3 = H$	16%
$R_1 = OH$, $R_2 = H$, $R_3 = Me$ (Adrenaline)	Ferricyanide	$R_1 = OH$, $R_2 = H$, $R_3 = Me$	62%
	Iodate	$R_1 = OH$, $R_2 = I$, $R_3 = Me$	65%
$R_1 = OH$, $R_2 = H$, $R_3 = Pr^i$ (Aludrine)	Ferricyanide	$R_1 = OH$, $R_2 = H$, $R_3 = Pr^i$	40%
	Iodate	$R_1 = OH$, $R_2 = I$, $R_3 = Pr^i$	20%
$R_1 = OH$, $R_2 = Me$, $R_3 = H$ (Corbasil)	Ferricyanide	$R_1 = OH$, $R_2 = Me$, $R_3 = H$	20% *
	Iodate		44%

* As quinone semicarbazone.

equivalent intermediate state of oxidation and the complexes should properly be regarded as derived from two molecules of the semiquinone.



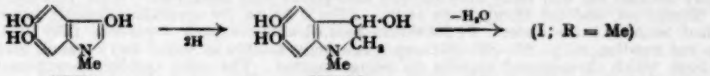
(X.)



(XI.)

Complexes of this kind could be formed from 2 : 3-dihydroindole-5 : 6-quinones and zinc ions as in the example (XI); in these cases the presence of the positive charges would render the complex water-soluble. Spontaneous decomposition could occur by release of protons from the 2- and 3-positions of a dihydroindole nucleus, resulting in isomerisation to a 5 : 6-dihydroxyindole.

Another method for the preparation of 5 : 6-dihydroxy-1-methylindole was investigated. This consisted of the reduction of 3 : 5 : 6-trihydroxy-1-methylindole (XII), easily obtained from adrenaline *via* adrenochrome, with Raney nickel–aluminium alloy and aqueous sodium hydroxide. Since it was found that the same reagents reduce indole to 2 : 3-dihydroindole,



(XII.) (XIII.) (I; R = Me)

the reaction must involve reduction to leuco-adrenochrome (XIII) followed by dehydration. That this dehydration occurs with remarkable ease has already been shown by Harley-Mason (*J.*, 1950, 1276).

Experimental.

Oxidation of β-3 : 4-Dihydroxyphenyl-N-methylalanine.—To a solution of the amino-acid (0·2 g.) and sodium hydrogen carbonate (0·1 g.) in water (5 c.c.), a solution of potassium ferricyanide (1·3 g.) and sodium hydrogen carbonate (0·3 g.) in water (15 c.c.) was added with stirring. After 10 minutes the deep red solution thus obtained was treated with 20% zinc acetate solution (8 c.c.), and the mixture shaken vigorously. During 5 minutes the red colour changed to grey, and a little sodium dithionite was added to prevent oxidation. The precipitated zinc ferrocyanide was filtered off and washed. The filtrate was extracted with peroxide-free ether (4 × 10 c.c.), the ethereal solution separated and dried over alkali-free sodium sulphate, and the solvent removed. Recrystallisation of the residual gum (benzene–light petroleum) gave 5 : 6-dihydroxy-1-methylindole (60 mg., 40%), m. p. 136°.

Similar oxidations were performed with β-3 : 4-dihydroxyphenylalanine in place of the *N*-methyl compound. In one experiment a yield of 30% of 5 : 6-dihydroxyindole, m. p. 140°, was obtained, but this could not be repeated and it appeared that the solid light brown residue obtained after removal of the ether was polymeric, and only very small amounts of the required product could be obtained from it by high-vacuum sublimation or solvent extraction.

Oxidation of L-β-3 : 4-Dihydroxyphenylalanine Ethyl Ester.—L-β-3 : 4-Dihydroxyphenylalanine (2·2 g.) was suspended in ethanol (50 c.c.) and saturated with dry hydrogen chloride without cooling. The solvent was removed under reduced pressure, and the residue dried in a desiccator over phosphoric oxide and potassium hydroxide, giving the ethyl ester hydrochloride as a glass which could not be crystallised. To a solution of the ester (2·2 g.) in water (10 c.c.), a saturated aqueous solution of potassium iodate (2·5 g.), followed by *n*-butanol (20 c.c.), was added. The mixture was gently agitated for about 40 minutes, during which the product crystallised out in the butanol layer, which was then separated and filtered, and the residue washed with water, methanol, and ether. *Ethyl 2 : 3-dihydro-3-iodoindole-5 : 6-quinone-2-carboxylate* (VI) (1·0 g.) formed red needles, m. p. 127° (decomp.), very sparingly soluble in water and slightly soluble in ethanol, giving a deep red solution. Light absorption in ethanol: $\lambda_{max.}$ = 2980 and 4900 A.; $\varepsilon_{max.}$ = 8330 and 1710 (Found: C, 38·5; H, 2·9; I, 36·7. $C_{11}H_{10}O_4NI$ requires C, 38·0; H, 2·9; I, 36·7%).

Isomerisation of the Iodo-quinone (VI).—(a) *Spontaneously.* A specimen of (VI) which had been kept for 6 months had become light brown. Fractional crystallisation from ethyl acetate–light petroleum gave *ethyl 5 : 6-dihydroxy-3-iodoindole-2-carboxylate* (VII) as small colourless plates, m. p. *ca.* 140° (decomp. and varying with rate of heating, with softening at 100°) (Found: C, 36·4, 36·9; H, 3·9, 3·2. $C_{11}H_{10}O_4NI,H_2O$ requires C, 36·2; H, 3·3%).

(b) *With alkali.* Addition of one drop of 2N-sodium hydroxide to a solution of (VI) in aqueous ethanol caused immediate decolorisation. The solution was at once acidified with acetic acid and extracted with ether. Removal of the solvent left a gum which gave (VII) on recrystallisation from ethyl acetate–light petroleum.

(c) *With zinc acetate.* When the quinone (VI) was stirred with 10% zinc acetate solution, the suspension of red crystals slowly became grey. Extraction with ether, filtration from dark undissolved material, and removal of the ether left a gum from which a small amount of (VII) was isolated by recrystallisation as above.

Reduction of (VI).—A solution of (VI) in aqueous ethanol was rapidly decolorised by addition of sodium dithionite. Extraction with ether and removal of the solvent left a gum which was recrystallised from ethyl acetate–light petroleum, giving *ethyl* 2 : 3-*dihydro*-5 : 6-*dihydroxy*-3-*iodoindole*-2-*carboxylate*, colourless needles, m. p. 103° (decomp.) (Found : C, 37·7; H, 3·4. $C_{11}H_{12}O_4NI$ requires C, 37·8; H, 3·4%); its ethanolic solution rapidly became red on exposure to air and the absorption spectrum was then identical with that of (VI).

1-(3 : 4-*Dihydroxyphenyl*)*prop*-2-*ylamine*.—A solution of veratraldehyde (8 g.), nitroethane (4 g.), and ammonium acetate (2 g.) in acetic acid (30 c.c.) was refluxed for 2 hours and then poured into water. The precipitated 1-(3 : 4-dimethoxyphenyl)-2-nitropropene formed yellow plates from ethanol, m. p. 73°.

The nitro-compound (6 g.) was placed in a small Soxhlet thimble and extracted under reflux into a solution of lithium aluminium hydride (4·1 g.) in ether (200 c.c.), boiling being continued for 6 hours. The resulting ethereal suspension was decomposed with a concentrated solution of sodium potassium tartrate, the ethereal layer separated and dried, and the solvent removed. The residual oil, 1-(3 : 4-dimethoxyphenyl)prop-2-ylamine (IV; R = Me), distilled at 95—97°/0·05 mm.

The amine (3 g.) was treated cautiously with hydrobromic acid (*d* 1·49; 10 c.c.) and refluxed 2 hours. The excess of acid was removed under reduced pressure and the residual gum crystallised on storage in a desiccator, giving the hydrobromide, which was washed with acetone.

Oxidation of Epinine.—(i) *With Potassium Iodate*. To a solution of epinine hydrochloride (0·5 g.) in water (30 c.c.) a saturated aqueous solution of potassium iodate (0·55 g.) was added. The solution rapidly became red and some brownish-red amorphous solid separated. After 10 minutes, this solid was filtered off, and the filtrate kept at 0°. After 6 hours, the crystalline deposit was collected and washed with a little water. 2 : 3-*Dihydro*-3-*iodo*-1-*methylindole*-5 : 6-*quinone* (IX) (0·4 g.) formed deep red needles, m. p. 85—87° (decomp.), sparingly soluble in water and ethanol, giving deep red solutions which decomposed rapidly on being warmed. The solid rapidly decomposed on storage (Found : C, 38·0; H, 3·0; I, 43·6. $C_9H_6O_2NI$ requires C, 37·4; H, 2·8; I, 44·0%).

The iodo-quinone (0·2 g.) was kept with a mixture of acetic anhydride (5 c.c.) and pyridine (5 c.c.) for 24 hours at room temperature, then poured into water; the precipitate was collected and twice recrystallised from aqueous methanol. 5 : 6-*Diacetoxy*-3-*iodo*-1-*methylindole* (VIII; R = Ac) formed colourless plates, m. p. 146—147° (Found : C, 42·3; H, 3·3. $C_{13}H_{12}O_4NI$ requires C, 41·8; H, 3·2%).

The iodo-quinone (0·2 g.) was stirred with 10% zinc acetate solution (5 c.c.); the red crystals were rapidly converted into a dark sticky mass, which was extracted with ether, the extract filtered, and the ether removed, leaving a yellow gum. Recrystallisation from benzene–light petroleum gave 5 : 6-*dihydroxy*-3-*iodo*-1-*methylindole* as yellow needles, m. p. 88—90° (decomp.) (Found : C, 37·1; H, 2·9. $C_9H_8O_2NI$ requires C, 37·4; H, 2·8%). The iodo-indole was unstable and rapidly decomposed in boiling solvents and in the solid state. It gave an intense blue colour with aqueous alkali.

(ii) *With potassium ferricyanide*. To a solution of epinine hydrochloride (0·8 g.) in water (50 c.c.) a solution of potassium ferricyanide (5·2 g.) and sodium hydrogen carbonate (1·7 g.) in water (50 c.c.) was added with stirring. After 10 minutes, 30 c.c. of a 20% solution of zinc acetate were added and the mixture was shaken vigorously. After 10 minutes the red colour had changed to grey. A little sodium dithionite was added to prevent oxidation, and the precipitated zinc ferrocyanide filtered off. The pale yellow filtrate was extracted with peroxide-free ether (4 × 30 c.c.), the ethereal extract dried (Na_2SO_4), and the ether removed. The residual gum was recrystallised from benzene–light petroleum, giving 5 : 6-dihydroxy-1-methylindole (0·45 g.), m. p. 136°.

Similar treatment of 2-(3 : 4-dihydroxyphenyl)ethylamine and 1-(3 : 4-dihydroxyphenyl)prop-2-ylamine gave brownish-red rather than red solutions on oxidation, and the ethereal extracts, although giving a positive Ehrlich reaction and showing a bluish-violet fluorescence indicating the presence of dihydroxyindoles, contained insufficient material for working up.

Reduction of 3 : 5 : 6-*Trihydroxy*-1-*methylindole*.—The trihydroxy-indole (1·0 g.) was dissolved in 10% sodium hydroxide solution (50 c.c.) and Raney nickel–aluminium alloy (5 g.) was added in small portions without cooling. The yellow colour slowly faded and the green fluorescence disappeared as the reaction proceeded. The supernatant liquor was decanted from undissolved metal and acidified with dilute sulphuric acid. The inorganic precipitate was filtered off, and the filtrate extracted with peroxide-free ether (4 × 20 c.c.). Removal of the solvent after drying (Na_2SO_4) gave a gum from which 5 : 6-dihydroxy-1-methylindole (0·4 g.), m. p. 135°, was isolated by recrystallisation from benzene–light petroleum.

Formation of Zinc Complexes from Oxidised Catechols.—To a filtered solution of catechol (1·1 g.) and zinc acetate (10 g.) in water (200 c.c.) a filtered aqueous solution of sodium persulphate (2·5 g.) was added. The solution rapidly became green and a very dark green precipitate was deposited. After 2 hours the precipitate was collected, well washed with water, and dried (P_2O_5). The *zinc* complex (Found : C, 52·2; H, 3·0; ZnO, 29·5. $C_{13}H_8O_4Zn$ requires C, 51·1; H, 2·8; ZnO, 28·8%) formed a very dark green powder insoluble in the common organic solvents with the exception of acetic acid, which gave an orange-red solution with apparent decomposition. It also dissolved with decomposition in aqueous mineral acids.

Similar treatment of 4-methylcatechol (1·2 g.) gave a similar *zinc* complex (Found : C, 56·1; H, 4·9; ZnO, 23·1. $C_{14}H_{12}O_4Zn$ requires C, 57·0; H, 4·75; ZnO, 24·0%).

UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, April 9th*, 1951.]

498. *Coenzyme A. Part II.* Evidence for its Formulation as a Derivative of Pantothenic Acid-4′ Phosphate.*

By J. Baddiley and E. M. Thain.

The behaviour of pantothenic acid-2′ and -4′ phosphates and the 2′ : 4′-diphosphate towards acid and alkali has been examined. This has facilitated the determination of hydrolysis conditions under which coenzyme A might be expected to give rise to such phosphates. Methods for the separation and detection on paper of these phosphates and related substances have been developed.

Mild alkaline or acid hydrolysis of coenzyme A gave a product indistinguishable on paper chromatography from pantothenic acid-4′ phosphate. The presence of both 2-mercaptoethylamine and adenosine-5′ phosphate residues is confirmed. Possible formulæ for the coenzyme are advanced.

The factor which participates in a number of biological acetylation processes, coenzyme A, has been shown from analyses of its highly active concentrates to be a derivative of pantothenic acid (Lipmann, Kaplan, Novelli, Tuttle, and Guirard, *J. Biol. Chem.*, 1947, **167**, 869). Recent investigations (De Vries, Govier, Evans, Gregory, Novelli, Soodak, and Lipmann, *J. Amer. Chem. Soc.*, 1950, **72**, 4838) have shown that besides pantothenic acid the molecule contains adenosine, a sulphur-containing substance probably related to cysteine, and two or three phosphate groups. A degradation product of coenzyme A, produced by the action of pigeon-liver extracts, was thought to be a phosphoric ester of pantothenic acid since it liberated pantothenic acid and phosphate on further enzymic breakdown (Novelli, Kaplan, and Lipmann, *J. Biol. Chem.*, 1949, **177**, 97). Although this degradation product has not yet been isolated and characterised it was thought that the correctness of these views could be checked by synthesis of the several possible pantothenic acid phosphates. As the liver degradation product was active in stimulating the growth of *Acetobacter suboxydans* (Novelli, Flynn, and Lipmann, *ibid.*, p. 493) a ready check of identity was available. In Part I of this series * we described the synthesis of pantothenic acid-2′ (I; R = PO_3H_2, R′ = H) and -4′ (I; R = H, R′ = PO_3H_2) phosphates, neither of which showed activity towards *A. suboxydans*. The two monophosphates,

R′O·CH_2·CMe_2·CH(OR)·CO·NH·$[CH_2]_2$·CO_2H (I.)

R′O·CH_2·CMe_2·CH(OR)·CO_2H (II.)

and the 2′ : 4′-diphosphate (I; R = R′ = PO_3H_2) were synthesised independently by King and Strong (*Science*, 1950, **112**, 562) using slightly different methods. These results, although in no way suggesting the absence of a pantothenic acid phosphate structure in coenzyme A, do indicate that the liver degradation product cannot be regarded as a simple monophosphate of pantothenic acid. Experiments described later in this paper eliminate the possibility of a pantothenic acid-2′ : 4′ diphosphate structure in coenzyme A itself and therefore also in the liver degradation product.

Observations made during the preparation of concentrates of "bound pantothenic acid" (*i.e.*, coenzyme A or related substances) from natural sources suggested that when in this bound form pantothenic acid is distinctly more stable to hydrolysis than is the free acid itself (Jukes, *Biol. Symposia*, 1947, **12**, 261; King, Fels, and Cheldelin, *J. Amer. Chem. Soc.*, 1949, **71**, 131; Neal and Strong, *ibid.*, 1943, **65**, 1659). A study of the stability of the amide bonds in our synthetic phosphates was undertaken in this connection. Alkaline hydrolysis was investigated, the extent of hydrolysis being measured by the amount of free amino-nitrogen, determined by Van Slyke's method (Peters and Van Slyke, "Quantitative Clinical Chemistry," 1932, Vol. II, p. 385), present in solution at given times. The results summarised in the Experimental section show that the amide bond in both phosphates is very much more stable to alkali than is that in pantothenic acid. In 0·5N-sodium hydroxide at 25° pantothenic acid hydrolysis obeyed first-order kinetics ($k = 1{\cdot}54 \times 10^{-4}$ sec.$^{-1}$), being 50% hydrolysed in 70 minutes. Under similar conditions the 2′-phosphate was 50% hydrolysed in 50 hours, but only 5% hydrolysis of the 4′-phosphate occurred in 24 hours. At 100°, however, appreciable

* The paper entitled "The Synthesis of Pantothenic Acid-2′ and -4′ Phosphates as Possible Degradation Products of Coenzyme A" by J. Baddiley and E. M. Thain (*J.*, 1951, 246) is regarded as Part I.

hydrolysis of the amide bonds in both phosphates occurred. First-order kinetics were not observed in either case. The rate for the 2′-phosphate was rapid at first (50% hydrolysed in about 10 minutes), but fell subsequently so that the time taken between 70% and 85% hydrolysis was 200 minutes. The 4′-phosphate was attacked smoothly but slowly, being only 20% hydrolysed in 330 minutes, a rate similar to the later stages of hydrolysis of the 2′-phosphate. The pantothenic acid amide bond in coenzyme A is about 30% hydrolysed in N-alkali at 100° in 180 minutes (King and Strong, *J. Biol. Chem.*, 1951, **189**, 325). This is in good agreement with our figures for pantothenic acid-4′ phosphate and differs quite markedly from those for the 2′-phosphate. The conclusion that a pantothenic acid-4′ phosphate structure may be present in the coenzyme is confirmed by observations described later in this paper.

As was shown in Part I (*loc. cit.*) the phosphate bonds in the synthetic phosphates are very stable to alkali and rather stable to acid. It seemed probable, then, that alkaline hydrolysis or even mild acid hydrolysis of coenzyme A should give rise to one of these phosphates if such a grouping were present in its molecule. According to the conditions necessary to hydrolyse other bonds in the coenzyme one may expect to obtain mixtures containing, not only a phosphate of pantothenic acid, but also a phosphate of pantoic acid (II) or its lactone both of which have lost the β-alanine residue. However, in view of the very small amounts of coenzyme A available to us for chemical study at this stage (a total of about 5 mg. was used in the work described in this paper), and also in view of the difficulties so far experienced in obtaining crystalline or otherwise characterisable derivatives of the phosphates of pantothenic and pantoic acid, the isolation of such degradation products and their chemical identification was not possible. Consequently, an investigation was made of the behaviour of these substances in paper chromatography, *n*-propyl alcohol–ammonia (Hanes and Isherwood, *Nature*, 1949, **164**, 1107) and *iso*butyric acid–ammonium *iso*butyrate–water (Magasanik, Vischer, Doniger, Elson, and Chargaff, *J. Biol. Chem.*, 1950, **186**, 37) proving satisfactory solvents. The phosphoric esters were detected as blue spots by spraying with perchloric acid and ammonium molybdate, followed by development with hydrogen sulphide (Hanes and Isherwood, *loc. cit.*). Adenine derivatives were also detected by inspection in ultra-violet light. The following were examined in both solvent systems : pantothenic acid-2′ and -4′ phosphate and -2′ : 4′ diphosphate, alkali hydrolysates of these, pantolactone-2 phosphate, adenylic acids a and b, adenosine-5′ phosphate and diphosphate, and inorganic phosphate.

Of the phosphates of pantothenic and pantoic acids the most rapidly moving in both solvent systems was pantolactone-2 phosphate. In *n*-propyl alcohol–ammonia this substance gave two clear spots, a fast-moving one which was shown to be the amide arising from the action of ammonia on the lactone and a slower one of the ammonium salt of pantoic acid-2 phosphate (II; R = PO_3H_2; R′ = H). After treatment of the lactone phosphate with ammonia, followed by paper chromatography, only one spot was observed, again corresponding with the amide, whereas pretreatment with barium hydroxide and then ammonium sulphate resulted in a spot of the ammonium salt of the acid. In the *iso*butyric acid system pantolactone-2 phosphate gave only one spot, the acidic conditions preventing opening of the lactone ring. Good separations of the pantothenic acid phosphates, both from each other and from adenosine-5′ phosphate, adenylic acids a and b, and adenosine diphosphate, were observed in both solvent systems. The relative positions of the pantothenic acid phosphates with respect to adenosine phosphates were reversed on changing from *n*-propyl alcohol–ammonia to *iso*butyric acid–ammonium *iso*butyrate. In neither system was it possible to effect more than partial separation of pantothenic acid-4′ phosphate from its product of alkaline hydrolysis [pantoic acid-4 phosphate (II; R = H; R′ = PO_3H_2)], although the very characteristic appearance of the double spot from pantolactone-2 phosphate was observed quite easily in hydrolysates of pantothenic acid-2′ phosphate.

The isomeric adenylic acids were separated readily on paper from the pantothenic acid phosphates. In *n*-propyl alcohol–ammonia all three were slower-moving than pantothenic acid-4′ phosphate. Isomers a and b (Carter and Cohn, *Fed. Proc.*, 1949, **8**, 190) were not separated in this system but moved slightly faster than adenosine-5′ phosphate. In *iso*butyric acid–ammonium *iso*butyrate the adenylic acid isomers moved considerably faster than pantothenic acid-4′ phosphate and, although isomers a and b were not separated, they moved noticeably faster than adenosine-5′ phosphate.

A mild alkali hydrolysate of coenzyme A from which inorganic phosphate had been removed by precipitation as its barium salt gave two phosphorus-containing spots from each of the two solvent systems. One corresponded in position with pantothenic acid-4′ phosphate or its hydrolysis product, pantoic acid-4 phosphate, and the other spot with adenosine-5′ phosphate.

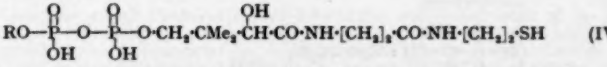
Shorter periods of hydrolysis sometimes resulted in the occurrence of an additional, slow-moving spot; this has not been identified yet but may represent a degradation product. The absence of purine derivatives at the positions occupied in both chromatograms by the pantothenic acid-4′ phosphate spots was ascertained by examination in ultra-violet light. The marked absence of spots corresponding to pantothenic acid-2′ phosphate, the 2′ : 4′-diphosphate, or their hydrolysis products eliminates the existence of such groupings in the coenzyme. In acid hydrolysates pantothenic acid-4′ phosphate was readily observed but little, if any, adenosine-5′ phosphate was present.

While these experiments should not be regarded as providing conclusive proof of the presence of a pantothenic acid-4′ phosphate residue in coenzyme A, at least they strongly suggest it. Confirmation should be forthcoming when more material is available and isolation of degradation products can be attempted. The presence of adenosine-5′ phosphate in coenzyme A hydrolysates confirms the findings of Lipmann (private communication). It was shown that no free adenosine-5′ phosphate was present in the sample of coenzyme before hydrolysis.

A phosphorus-free derivative of pantothenic acid has been isolated recently from natural sources (McRorie, Masley, and Williams, *Arch. Biochem.*, 1950, **27**, 471; Brown, Craig, and Snell, *ibid.*, p. 473) and shown to stimulate the growth of *Lactobacillus bulgaricus* (*Lactobacillus bulgaricus* factor, LBF). It has been proved by synthesis to be (III) (Snell, Brown, Peters,

$$HO{\cdot}CH_2{\cdot}CMe_2{\cdot}CH(OH){\cdot}CO{\cdot}NH{\cdot}[CH_2]_2{\cdot}CO{\cdot}NH{\cdot}[CH_2]_2{\cdot}SH \quad \text{(III.)}$$

Craig, Wittle, Moore, McGlohon, and Bird, *J. Amer. Chem. Soc.*, 1950, **72**, 5349). An enzymic degradation product of coenzyme A was indistinguishable from (III) in its ability to stimulate the growth of this organism, hence a close chemical relationship between coenzyme A and (III) can be assumed. If this degradation product of the coenzyme is the 2-mercaptoethylamide (III) of pantothenic acid, then the " cystein-like " substance described by De Vries *et al.* (*loc. cit.*) as a component of the coenzyme should be 2-mercaptoethylamine. An acid hydrolysate of coenzyme A was neutralised and subjected to steam-distillation on a micro-scale. The distillate contained a substance which gave both a ninhydrin and a nitroprusside reaction. Paper chromatography in butyl alcohol–acetic acid–water showed that these two reactions were given by a single substance corresponding in position on the paper with synthetic 2-mercaptoethylamine. This confirms the close relationship between (III) and coenzyme A. Synthetic 2-mercaptoethylamine was prepared for these studies by a convenient modification of Gabriel's method (*Ber.*, 1889, **22**, 1137; 1891, **24**, 1110) involving the hydrolysis of *N*-2-phthalimidoethylthiuronium bromide.



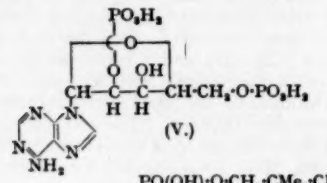
(IV.)

Novelli, Kaplan, and Lipmann (*Fed. Proc.*, 1950, **9**, 209) have shown that the coenzyme is probably a dinucleotide since it is split by a dinucleotidase. Its marked alkali lability is also consistent with this view. On the basis of this and the foregoing evidence we have proposed formula (IV) for coenzyme A (Baddiley and Thain, *Chem. and Ind.*, 1951, 337). The group R is adenosine, possibly bearing an additional phosphate group. If coenzyme A contains only two phosphate groups per molecule, then the position of attachment of the pyrophosphate group to the adenosine residue would be 5′, as indicated by the presence of adenosine-5′ phosphate in hydrolysates of the coenzyme. If three phosphate groups are present, however, the situation is less clear. It is very unlikely that an additional phosphate group could be attached at position 2′ in the pantothenic acid residue since we would expect then to detect pantothenic acid-2′ : 4′ diphosphate in coenzyme hydrolysates, both phosphate groups in this diphosphate being stable under the hydrolytic conditions we employed. There remain positions 2′ and 3′ in the adenosine residue as the most probable points of attachment of an additional phosphate group. Although it is not possible to differentiate at this stage between these, we favour 2′ by analogy with the formula recently proposed for coenzyme II (triphosphopyridine nucleotide) (Kornberg and Pricer, *J. Biol. Chem.*, 1950, **186**, 557). In this connection it would be of interest to study the nature of the products of dinucleotidase action on coenzyme A. If one product were adenosine-2′ : 5′ diphosphate (V), then the above formal relation between the two coenzymes would be established. The failure to detect formation of adenosine-5′ phosphate from coenzyme A by the action of a dinucleotidase (Lipmann, Kaplan, Novelli, Tuttle, and Guirard, *ibid.*, p. 235) would be explained in this way. King and Strong (*ibid.*, 1951, **189**, 325), contrary to the findings of Novelli, Kaplan, and Lipmann (*loc. cit.*),

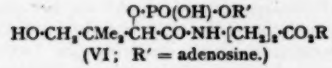
observed only a small destruction of activity when coenzyme A was treated with a dinucleotidase. They concluded that a pyrophosphate linkage was not present and suggested a partial formula (VI) containing a disubstituted monophosphate grouping. Now the phosphate bond in the adenosine phosphates is considerably less stable to alkali than that in pantothenic acid-4′ phosphate. Consequently, if in the structure postulated by King and Strong the phosphate linkage involved only the 4′-position in pantothenic acid and the 5′-position in adenosine, then hydrolysis should give rise to pantothenic acid-4′ phosphate and adenosine, not as we have observed in coenzyme A hydrolysates adenosine-5′ phosphate. (Their assignation of the phosphate to the 2′-position in the pantothenic acid moiety is presumably arbitrary and, as we have shown, incorrect.) However, a structure (VII) would be expected to give on hydrolysis both pantothenic acid-4′ phosphate and adenosine-5′ phosphate and would be unaffected by dinucleotidase action. Final decision between formulæ (IV) and (VII) for coenzyme A must await further investigation. The position of attachment of the pantothenic acid phosphate part to the adenosine-5′ phosphate in (VII) is still undecided.

The evidence presented so far does not exclude the possibility of cyclic phosphate structures for coenzyme A. Such cyclic phosphates of pantothenic acid are not known, consequently it is not possible to predict their behaviour under the hydrolytic conditions of our experiments or their behaviour on paper chromatography.

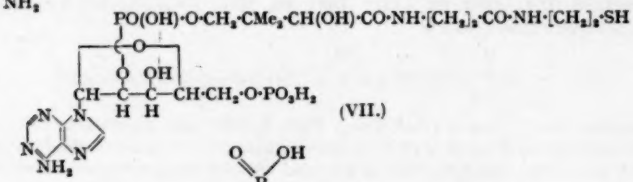
The structure of the liver degradation product is still uncertain. The inability of pantothenic acid-4′ phosphate to stimulate the growth of *A. suboxydans* might be interpreted in one of two ways. First, this degradation product may contain a cyclic phosphate [*e.g.*, (VIII)], which would suggest the presence of such a group in coenzyme A, or, secondly, it may be a derivative of (III) bearing a phosphate group on the primary hydroxyl group. The synthesis of these phosphates is under investigation.



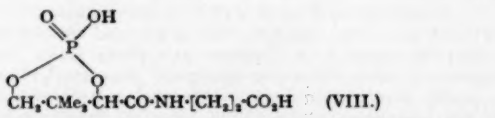
(V.)



(VI; R′ = adenosine.)



(VII.)



(VIII.)

Lynen and Reichert (*Angew. Chem.*, 1951, **63**, 47) have suggested that the acetylating activity of coenzyme A involves the terminal thiol group. They suggest that the coenzyme accepts an acetyl group at this position and transfers it to the substrate. In its mercaptoacetyl form coenzyme A presumably contains its acetyl in an "energy-rich" state. Lynen and Reichert provide evidence that such an acetyl group is present in their coenzyme A preparations obtained from yeast. Insufficient material has been available to us for acetyl determinations on coenzyme A prepared from *Streptomyces fradiae* as described by De Vries *et al.* (*loc. cit.*). It would seem reasonable, however, to expect a variable acetyl content depending to some extent on the metabolic state of the tissues or organism at the time of isolation. The expected lability of such an acetyl group might result in the isolation of a coenzyme A which, although highly active in acetylation systems, does not itself contain any acetyl group.

The experiments described in this paper were performed on a sample of coenzyme A with an acetylating activity of 180 units/mg., as measured by its capacity to acetylate sulphanilamide (Kaplan and Lipmann, *J. Biol. Chem.*, 1948, **174**, 37). Analytically pure samples of the coenzyme have not yet been described and until such are available it is not possible to rule

out the occurrence of hitherto unidentified groups in the molecule. The possible occurrence of more than one coenzyme A must also be considered. In this connection we have shown that paper chromatography of our sample of the coenzyme showed the presence of two rather slow-moving phosphate fractions, both of which were active in the acetylation of sulphanilamide. The total recovery of activity from the paper was low, however, and the method is as yet insufficiently refined for the isolation and chemical investigation of the different fractions.

Experimental.

Hydrolysis of the Amide Bond in Pantothenic Acid and Pantothenic Acid Phosphates.—Calcium pantothenate (13 mg.) and the barium salts of pantothenic acid-2′ and -4′ phosphates (25 mg.) were severally dissolved in 0·5N-sodium hydroxide (10 ml.), and the solution kept at 25° (thermostat). Aliquots (1 ml.) were analysed for free β-alanine by Van Slyke's method (*loc. cit.*).

Pantothenic acid.

Time (min.)	15	47	75	106	152	232	307
Hydrolysis, %	12·5	37·8	51·8	59·2	71·7	86·2	93·1

Pantothenic acid-2′ phosphate.

Time (hr.)	2	5	7	29·2	31·5	49·6
Hydrolysis, %	4·8	9·2	11·3	40·9	42	52·7

Under similar conditions pantothenic acid-4′ phosphate was only 5% hydrolysed after 24 hours.

A plot of log (decay) against time derived from these figures gave a straight line for pantothenic acid indicating first-order kinetics, $k = 1{\cdot}54 \times 10^{-4}$ sec.$^{-1}$ at 25°, but a curve was obtained for pantothenic acid-2′ phosphate probably owing to simultaneous hydrolysis of the phosphate complicating the rate of hydrolysis.

In view of the slow hydrolysis of the pantothenic acid phosphates the temperature was increased to 100°, conditions otherwise being the same, and smooth curves were obtained confirming the greater stability of pantothenic acid-4′ phosphate.

Pantothenic acid-2′ phosphate.

Time (min.)	5	10	15	38	70	80	140	270
Hydrolysis, %	28·7	51·0	55·0	67·1	69·9	75·2	80·1	89·0

Pantothenic acid-4′ phosphate.

Time (min.)	37	80	120	240	330
Hydrolysis, %	4	7·4	8·1	15·5	19·5

Hydrolysis of Coenzyme A and Pantothenic Acid Phosphates, and Examination of the Products by Paper Chromatography.—Coenzyme A, adenosine-5′ phosphate, pantolactone-2 phosphate, and the barium salts of pantothenic acid phosphates (*ca.* 0·5 mg.) were severally heated in sealed tubes with N-hydrochloric acid (0·1 ml.) or 0·3N-barium hydroxide (0·1 ml.) at 100° for 0·5—2 hours.

In acid hydrolyses the solutions were evaporated in a vacuum and the residues dissolved in 0·3N-barium hydroxide (0·1 ml.) to precipitate any free phosphate; thereafter acid and alkaline hydrolyses were treated similarly. Carbon dioxide was passed through the solutions until precipitation was complete and the solutions were boiled for 1—2 minutes to destroy any barium hydrogen carbonate. Barium carbonate was removed by centrifugation and sufficient ammonium sulphate added to precipitate barium from soluble barium salts. Barium sulphate was removed by centrifugation and the solutions evaporated in a vacuum.

The dried residues were dissolved in small volumes of water, usually about 0·05 ml.; then sufficient of these solutions to contain about 10 μg. of phosphorus was chromatographed on Whatman's No. 1 filter paper with (*a*) *n*-propyl alcohol–ammonia–water (6 : 3 : 1) (Hanes and Isherwood, *loc. cit.*) and (*b*) *iso*butyric acid–ammonium *iso*butyrate–water (*iso*butyric acid, 10 vols.; 0·5N-aqueous ammonia, 6 vols.) (Magasanik *et al.*, *loc. cit.*). Washing the paper with acids was unnecessary in the chromatography of phosphate esters of pantothenic acid since there was no serious tailing of the spots. The only advantage of washing was the increased speed of running, which had little effect on the observed R_F values. Since no special temperature control was employed the R_F values varied within small limits. Those given below are for room temperature (*ca.* 20°) and are averages of many runs.

Phosphates were detected by perchloric acid–molybdate spray (Hanes and Isherwood), and adenine derivatives by their fluorescence in ultra-violet light.

When the hydrolysis had been carried out in glass tubes a " ghost spot " appeared in the same place on the paper, probably due to silicates, irrespective of the nature of the substance hydrolysed or even whether any phosphate was being hydrolysed at all. These spots appeared a day or two after the papers had been developed and thus were readily distinguished.

The synthetic-2′ and -4′ phosphates of pantothenic acid were found to be homogeneous, but some samples of the -4′ phosphate gave in addition to the main spot a much weaker, faster-moving one (R_F 0·8 in *n*-propyl alcohol–ammonia), probably due to a small amount of material from which the benzyl

or phenyl groups had been only partly removed by hydrogenolysis. Pantothenic acid diphosphate prepared by King and Strong's method (*loc. cit.*) contained small amounts of pantolactone phosphate.

Substance.	PrOH–NH_3. R_F.	Pr^i–CO_2H–NH_3. R_F.
Pantolactone-2 phosphate	0·55 (amide) 0·38 (NH_4 salt)	0·57
Pantothenic acid-2′ phosphate	0·45	0·40
,, -4′ ,,	0·33 0·36 hydrol.	0·37
,, diphosphate	0·13	0·18
Adenosine-5′ phosphate	0·26	0·44
Adenylic acid a	0·30	0·56
,, b	0·30	0·56
Coenzyme A	0·07, 0·13	—
Adenosine diphosphate	0·18	—
Adenosine	0·73	0·79
Adenine	0·65	0·90

Detection of 2-Mercaptoethylamine in Coenzyme A.—Coenzyme A (1·0 mg.) was hydrolysed with 5N-hydrochloric acid (0·2 ml.) at 100° for 3 hours (sealed tube). The hydrolysate was evaporated in a vacuum, the residue dissolved in water (0·5 ml.) containing sodium sulphite (*ca.* 1 mg.), the pH adjusted to 8 (Na_2CO_3), and the mercaptoethylamine steam-distilled in a gentle stream of nitrogen. The distillate was acidified with acetic acid and evaporated in a vacuum, and the dried residue divided into two parts and run on paper in butyl alcohol–acetic acid–water together with an authentic sample of 2-mercaptoethylamine. The dried paper was divided and one half sprayed with ninhydrin, and the other with sodium sulphite–cyanide–nitroprusside. Spots were recorded with both sprays corresponding to 2-mercaptoethylamine, $R_F = 0{\cdot}26$.

Biological Tests.—Two samples of coenzyme A, one of which weighed 0·225 mg. (38 units), were run down a paper chromatogram with *n*-propyl alcohol–ammonia for 40 hours. The paper was dried and divided down the centre, and the unweighed sample developed for phosphate with the perchloric acid–molybdate reagent. Portions of paper corresponding to the two main spots on the developed chromatogram, which were 7 cm. apart, were cut out of the paper containing the weighed sample of coenzyme A and eluted with water for 3 hours, together with a portion of paper taken from between the two spots, it having been observed that there was a very faint spot at this position. The eluates were evaporated in a vacuum and the residues analysed for coenzyme A activity by Kaplan and Lipmann's method (*loc. cit.*). The fastest-moving spot contained 7 units, the slowest 0·8 unit, while a slight activity of 0·6 unit was observed in the middle spot (this however may have arisen from "lagging" of the active fast spot).

N-2-*Phthalimidoethylthiuronium Bromide.*—A solution of *N*-2-bromoethylphthalimide (101 g.) and thiourea (35 g.) in absolute alcohol (250 c.c.) was refluxed for 4½ hours. The solid which separated was filtered off from the cooled mixture, washed with a little cold alcohol, and dried. The *thiuronium* salt (104 g.) was sufficiently pure for subsequent conversion into 2-mercaptoethylamine. Recrystallised from alcohol it formed prisms, m. p. 243° (Found: C, 39·9; H, 3·5; N, 12·5. $C_{11}H_{12}O_2N_3SBr$ requires C, 40·0; H, 3·6; N, 12·7%).

N-2-*Phthalimidoethanethiol.*—To the thiuronium salt (25 g.), dissolved in the minimum amount of boiling water, a solution of sodium hydroxide (3·1 g.) in water (50 c.c.) was added. Boiling was continued for 5 minutes. The oil which separated solidified on cooling. The solid was filtered off, washed with water, and dried in a desiccator (13 g.). Recrystallised from aqueous alcohol it had m. p. 76°. Gabriel (*loc. cit.*) records m. p. 79—80° for *N*-2-phthalimidoethanethiol prepared by the action of potassium hydrogen sulphide on *N*-2-bromoethylphthalimide. 2-Mercaptoethylamine was prepared by hydrolysis of the thiol in hydrochloric acid according to Gabriel's method.

We thank Dr. F. Lipmann for a gift of coenzyme A, and Professor A. R. Todd, F.R.S., for samples of adenylic acid a and b. Thanks are offered also to Roche Products Ltd., and Imperial Chemical Industries Limited, Dyestuffs Division, for gifts of chemicals, and the Department of Scientific and Industrial Research for a Special Research Grant.

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499. *The Constitution of Barley Starch.*

By I. C. MacWilliam and E. G. V. Percival.

Barley starch has been found to contain 19% of amylose, a highly purified specimen of which was obtained by repeated precipitation with butanol. Methylation studies revealed that the amylose consisted of unbranched chains containing an average of *ca.* 400 glucose units. No amylopectin fraction was obtained free from amylose. The purest (containing 2% of amylose) was obtained by precipitation with aqueous pyridine saturated with butanol. The presence in the amylopectin of one non-reducing terminal group for 26 ± 2 non-terminal glucose residues was found by the methylation technique. This result was confirmed by periodate oxidation which also indicated that over 86% of the branching linkages between the unit chains involve 1 : 6-linkages.

The structures of various starches have been the subject of recent study (Brown, Halsall, Hirst, and Jones, *J.*, 1948, 27; Hirst, Jones, and Roudier, *J.*, 1948, 1779; Potter and Hassid, *J. Amer. Chem. Soc.*, 1948, **70**, 3488, 3774; Potter, Hassid, and Joslyn, *ibid.*, 1949, **71**, 4075; Meyer *et al.*, *Helv. Chim. Acta*, 1940, **23**, 845, 854, 865). In the present paper these investigations have been extended to the starch from the barley grain (" Pioneer " variety). The starch was separated by a purely mechanical process and the use of reagents likely to cause degradation was avoided.

The amylose content of the starch was 19% as determined by the iodine potentiometric method (Bates, French, and Rundle, *J. Amer. Chem. Soc.*, 1943, **65**, 142; Wilson, Schoch, and Hudson, *ibid.*, p. 1381). Barley starch is, therefore, similar to most other cereal starches. A separation of the amylose and amylopectin components of the starch was carried out by four different methods (see Tables I, I*a*) as follows, with (1) thymol (Haworth, Peat, and Sagrott, *Nature*, 1946, **157**, 19; Bourne, Donnison, Haworth, and Peat, *J.*, 1948, 1687), (2) *n*-butanol (Schoch, *J. Amer. Chem. Soc.*, 1942, **64**, 2957; Higginbotham and Morrison, *Chem. and Ind.*, 1947, **25**, 45; *Shirley Inst. Mem.*, 1948, **22**, 141), (3) pyridine (*idem*, *loc. cit.*), and (4) 20% aqueous pyridine saturated with butanol (*idem*, *loc. cit.*). The results (Table I) show that in no case was fractionation complete. In all the fractionations described (Table I) the " amylose " fraction generally contained 25—40% of amylopectin, and the " amylopectin " fraction contained 5—8% of amylose. It, therefore, appeared that no single method of fractionation was capable of yielding pure amylose and amylopectin from barley starch. Reprecipitations of the " amylose " fraction with thymol failed to effect further purification but by repeated precipitation with butanol (*idem*, *loc. cit.*), with low concentrations of amylose (0·2%), fractions were obtained which took up 20·9% of their own weight of iodine. Further reprecipitation (amylose concentration, 0·1%) failed to raise this figure. Since precipitation with pyridine (Table I) yielded the " amylose " fraction containing the greatest proportion of the total amylose a larger quantity of starch was fractionated by this method. The crude amylose was then (Table II) reprecipitated several times with butanol (amylose concentration 0·2%). The product was finally obtained in good yield (representing about 80% of the total amylose present in the starch) and took up 20·9% of its own weight of iodine (Table II). Further reprecipitation (0·1% concentration) failed to raise this value which compares favourably with those found by Higginbotham and Morrison (*loc. cit.*) for other amyloses. On examination under the microscope the amylose–butanol complexes appeared similar to those already described for such complexes from other starches, and it is concluded that the barley amylose–butanol complex is approaching its purest state when it separates in the form of short needles (cf. Kerr, " Chemistry and Industry of Starch," New York 1944, p. 147).

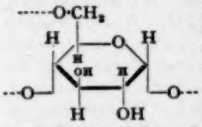
The barley amylose was methylated by repeated treatments with methyl sulphate and sodium hydroxide in nitrogen, to give a product containing OMe, 44·7% (calc. : OMe, 45·6%). Previous workers have detected small quantities of tetramethyl glucose (or tetramethyl methylglucoside) in the hydrolysates of methylated amyloses. Meyer, Wertheim, and Bernfeld (*Helv. Chim. Acta*, 1940, **23**, 865; 1941, **24**, 378) estimated that the chain lengths of maize and potato amyloses were 200—300 units. Similar results were found by Hess and Krajnc (*Ber.*, 1940, **73**, 976) and Hassid and McCready (*J. Amer. Chem. Soc.*, 1943, **65**, 1157) for potato amylose. Brown, Halsall, Hirst, and Jones (*loc. cit.*), estimated sago amylose to have a chain length of 250 ± 100 glucose units, and Bourne, Fantes, and Peat (*J.*, 1949, 1109) and Barker, Bourne,

and Wilkinson (*J.*, 1950, 3027) found chain lengths of about 200 glucose units for samples of potato amylose.

The hydrolysed methylated barley amylose was separated on a cellulose column (Hough, Jones, and Wadman, *J.*, 1949, 2511). The main product of hydrolysis was identified as 2 : 3 : 6-trimethyl D-glucose. This was accompanied by small quantities of tetramethyl glucose and a mixture of dimethyl glucoses. The amount of tetramethyl glucose was estimated to be 0·24—0·29% of the methylated glucoses produced on hydrolysis, corresponding to the presence of one non-reducing terminal group per 400 ± 40 glucose residues. Paper chromatography indicated the presence of at least two different dimethyl glucoses corresponding (1) to 2 : 3-dimethyl glucose and (2) to 2 : 6- and/or 3 : 6-dimethyl glucose (cf. Bell, *J.*, 1948, 992; Hirst, Hough, and Jones, *J.*, 1949, 928; Barker, Bourne, and Wilkinson, *loc. cit.*, for methylated starches and amylopectins). The dimethyl glucoses amounted to *ca.* 1·6% of the total hydrolysate and the proportion of 2 : 3-dimethyl glucose was estimated to be only *ca.* 0·05%. The mixture of dimethyl sugars was converted into the methylglycosides and the amount of 2 : 6-dimethyl methylglucoside was estimated to be *ca.* 1·3% by oxidation with sodium metaperiodate (Bell, *loc. cit.*). These dimethyl glucoses do not appear to have any structural significance for the reasons outlined below.

Attempts to determine the molecular size of barley amylose itself have given somewhat conflicting results. Oxidation with potassium periodate (Brown, Halsall, Hirst, and Jones, *loc. cit.*) and titration of the formic acid released, indicated the production of one mole of formic acid per 72 ± 7 units. If it is assumed that two molecules of formic acid are released from the reducing end-group, this result corresponds to the presence of one non-reducing terminal group per 216 ± 20 glucose residues. The colorimetric method of estimating the reducing power, in which 3 : 5-dinitrosalicylic acid is used in alkaline solution (Meyer, Noelting, and Bernfeld, *Helv. Chim. Acta*, 1948, **31**, 103) and the intensity of the colour is compared with that developed by maltose solutions of known concentration, indicated the presence of one aldehyde group per 135 ± 20 glucose units.

After hydrolysis of the amylose oxidised by periodate and examination by paper chromatography, no glucose was observed to be present. This indicated that there were in the amylose no glucose residues joined to each other through $C_{(2)}$ and $C_{(3)}$ (see Hirst, Jones, and Roudier, *loc. cit.*). All residues must, therefore, be joined through $C_{(1)}$, $C_{(4)}$, or $C_{(6)}$. Since the hydrolysed methylated derivative yielded only a very small proportion of 2 : 3-dimethyl glucose it is concluded that the greater part of the amylose consists of straight chains of glucose units united by 1 : 4-α-linkages. The appearance of 2 : 6- and 3 : 6-dimethyl glucoses in the hydrolysate must, therefore, be due to incomplete methylation of the polysaccharide or to the demethylation of 2 : 3 : 6-trimethyl glucose, and the presence of the small amount of 2 : 3-dimethyl glucose detected may also be attributed to these causes. From the available evidence, it is justifiable to consider that barley amylose consists for the most part of long unbranched chains of D-glucose residues united by 1 : 4-α-linkages. It is not yet possible to assign a precise length to the chain although it is certainly of the order of several hundred glucose units.



The fractionations employed (Table I) did not yield an amylopectin fraction containing less than 5% of amylose. The subjection of these fractions to further treatment with the precipitants did not yield any significant amounts of the amylose complex.

In their studies on fractionations of starches, Higginbotham and Morrison (*loc. cit.*) isolated only one sample of amylopectin which was free from amylose. This was obtained by dispersing the starch in water containing pyridine (20% by volume) followed by saturation with *n*-butanol; the yield of the non-precipitated fraction was low, however, since as much as 50% of the total amylopectin was precipitated with the amylose. Fractionation of barley starch by this method yielded an " amylopectin " fraction containing only 2% of amylose but representing only 50% of the total amylopectin present in the starch (cf. Table I). This result seemed to indicate that in barley starch, as in other starches, there exists a portion of the amylose which is not readily separated from the amylopectin.

The " amylopectin " (containing 2% of amylose) was methylated by repeated treatments with methyl sulphate and sodium hydroxide followed by methyl iodide and silver oxide. The methylated product (OMe, 45·0%) was hydrolysed and, by filter-paper chromatography, the presence of the following sugars was indicated: (*a*) 2 : 3 : 4 : 6-tetramethyl glucose (4·2%), (*b*) 2 : 3 : 6-trimethyl glucose (90·5%), (*c*) dimethyl glucose (5·3%); and (*d*) traces of monomethyl glucose and of glucose. The proportion of tetramethyl glucose corresponds to the

presence of one non-reducing terminal group per 25 glucose residues. A similar result was obtained when a quantity of the methylated amylopectin hydrolysate was separated on a cellulose column (Hough, Jones, and Wadman, *loc. cit.*) although two distinct dimethyl glucoses were produced. From the amount of tetramethyl glucopyranose isolated (*ca.* 4%) it was calculated that the amylopectin contained one non-reducing terminal group per 26 ± 2 glucose units. The proportion of glucose units per non-reducing terminal group in barley amylopectin is, therefore, similar to those found for other amylopectins (see Brown, Halsall, Hirst, and Jones, *loc. cit.*). The yields of both the end-group and 2 : 3 : 6-trimethyl glucose (*ca.* 90%) could not have been seriously affected by losses caused by demethylation during the acid hydrolysis (cf. Bell, *loc. cit.*). A similar experiment carried out on chromatographically pure 2 : 3 : 4 : 6-tetramethyl glucose and 2 : 3 : 6-trimethyl glucose showed (paper chromatography) that the former gave 1% of trimethyl glucose whilst the latter gave 0·7% of dimethyl glucoses.

The 2 : 3 : 4 : 6-tetramethyl, 2 : 3 : 6-trimethyl, and 2 : 3-dimethyl glucoses (*ca.* 4·5%) were obtained crystalline. The tetramethyl glucose was identified as the crystalline anilide, the trimethyl glucose by its inversion of optical rotation in cold methanolic hydrogen chloride solution (Irvine and Hirst, *J.*, 1922, **121**, 1213), and the 2 : 3-dimethyl glucose as the crystalline 2 : 3-dimethyl gluconophenylhydrazide (Evans, Levi, Hawkins, and Hibbert, *Canad. J. Res.*, 1942, **20**, *B*, 175). The second dimethyl glucose fraction (*ca.* 1%) did not crystallise but the inversion of the optical rotation in cold methanolic hydrogen chloride solution indicated that the hydroxyl groups on $C_{(4)}$ and $C_{(3)}$ were free. Treatment of the mixture of derived methyl glycosides with sodium metaperiodate by Bell's method (*loc. cit.*) indicated that this fraction contained 79% of 2 : 6-dimethyl glucose in admixture with 3 : 6-dimethyl glucose.

Oxidation of the barley amylopectin by potassium periodate indicated the presence of one non-reducing terminal group per 24 glucose residues. Similar oxidation of the whole starch indicated the presence of one non-reducing terminal group per 30 glucose residues. Since the starch contains 19% of amylose, it can be calculated from this result that the amylopectin fraction contains one non-reducing terminal group per 24 glucose residues which agrees with the result calculated from the experiment with amylopectin itself.

Estimations of the reducing power of the amylopectin with 3 : 5-dinitrosalicylic acid did not yield significant results. This, together with other evidence, indicates that the amylopectin of barley has a high molecular weight and a highly branched structure. The presence of 2 : 3-dimethyl glucose in amount only slightly greater than the yield of tetramethyl glucopyranose indicates that branching takes place through $C_{(6)}$ of one of the 1 : 4-linked glucose residues of a unit chain, and additional evidence has been found as in the experiments on acorn starch (Hirst, Jones, and Roudier, *loc. cit.*) to support this view. On hydrolysis of the barley amylopectin after periodate oxidation the free glucose released (0·59%) indicated that at least 86% of the linkages between the unit chains were 1 : 6-linkages. It was not possible to decide whether in fact all the branch linkages were of this type or whether the remainder were 1 : 3- or, less probably, 1 : 2-junctions because the residual glucose might have arisen by incomplete oxidation, and the 2 : 6- and 3 : 6-dimethyl glucoses by a combination of incomplete methylation and demethylation.

Experimental.

Preparation of Starch.—Barley (Pioneer variety; 1 kg.) was roughly ground in a "Raymond" laboratory mill and extracted with light petroleum (b. p. 40—60°) to remove fatty substances. After being dried at a low temperature, the grist was shaken with water (5 l.) for 6 hours. Most of the fibrous material was removed from the suspension by passage through 40- and 150-mesh sieves. The screened suspension was set aside overnight and a deposit of material rich in starch settled out. The supernatant liquid was siphoned off and fresh water added to *d* 1·030 (by hydrometer). After being stirred for 1 hour, the suspension was centrifuged (1500 r.p.m.). The deposit consisted of two well-defined layers—the lower of almost pure starch and the upper of fine fibrous material together with insoluble protein and some associated starch. The upper layer was removed as completely as possible by scraping with a spatula and by washing with a stream of water. Water was added to the remaining starch layer (to give *d* 1·030) and the process was repeated until all traces of impurity were removed. The starch was filtered off, dried at 40°, and ground with pestle and mortar. The fat content was further reduced by boiling the starch under reflux with methanol containing water (15% by weight). After three such treatments, the starch was filtered off, washed with water, dried at 40°, and reground.

The white powder (137 g.) was observed to be composed of granules of a moderate size (10—35 μ.) and ovoid to spheroidal. A suspension of the starch in water gave a deep blue colour with iodine. On 7 hours' heating with boiling 2% sulphuric acid (250 parts), the starch (1 part) gave 96% of the theoretical amount of glucose. No visible residue was left after hydrolysis. No other sugar could be detected on the paper chromatogram. This material had $[\alpha]_D^{18}$ +157° (*c*, 1·0 in N-sodium hydroxide), $[\alpha]_D^{18}$ +188° (*c*, 0·69 in 30% perchloric acid) (Nielsen, *Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 176) [Found: sulphated ash, 0·2; N, 0·04% (Kjeldahl)]. The blue value determined by Hassid and McCready's method

(*J. Amer. Chem. Soc.*, 1943, **65**, 1154) as modified by Bourne, Haworth, Macey, and Peat (*J.*, 1948, 924) was 0·27—0·28 (potato starch under similar conditions gave a blue value of 0·28).

Potentiometric Determination of Amylose.—The method of Bates, French, and Rundle (*J. Amer. Chem. Soc.*, 1943, **65**, 142), as modified by Wilson, Schoch, and Hudson (*ibid.*, p. 1381), was used. The amount of iodine taken up by 1 g. of starch was 0·0402 g. If 21·5% is the amount of iodine taken up by pure amylose (Higginbotham and Morrison, *loc. cit.*), the amylose content of the sample of barley starch was 18·7%.

Acetylation of Barley Starch.—Pacsu and Mullen's method (*J. Amer. Chem. Soc.*, 1941, **63**, 1487) was used. Air-dried starch (15 g.) gave a chloroform-soluble acetate (21·5 g.), η_{sp}^{20}/c 1·13 (*c*, 0·4 in *m*-cresol), $[\alpha]_D^{18}$ +170° (*c*, 0·5 in chloroform) (Found: $CH_3{\cdot}CO$, 43·3. Calc. for $C_{12}H_{16}O_8$: $CH_3{\cdot}CO$, 44·8%).

The acetyl groups were removed by the method of Zemplén and Pacsu (*Ber.*, 1929, **62**, 1613), using sodium methoxide. Acetylated starch (10 g.) was dissolved in dry chloroform (250 c.c.). Dry methanol (50 c.c.) containing dissolved sodium (50 mg.) was slowly added with shaking. The mixture was shaken for 1 day and set aside for 2 days more. The fine white precipitate was filtered on a G4 sintered-glass filter and washed with several portions of ethanol until free from sodium, and finally with ether. This material (5·5 g.) had blue value (see above) 0·283 and $[\alpha]_D^{18}$ +192° (*c*, 0·6 in 30% perchloric acid) (Found: $CH_3{\cdot}CO$, nil). The sample was later used in the fractionation experiments (see Table I below, Method I*b*).

"Amylose" fraction.

Separation of Amylose.—Method I. By thymol. (*a*) The standard procedure of Bourne, Donnison, Haworth, and Peat (*loc. cit.*) for potato starch was applied though on a smaller scale (5—10 g.). Results (Table I) seemed to indicate that dispersion of the starch was incomplete. Various modifications, (*b*), (*c*), and (*d*) (Table I), were then applied to effect better dispersion.

Method II. By butanol (Schoch, *loc. cit.*; Higginbotham and Morrison, *loc. cit.*). Dry starch (5 g.), suspended in cold water (20 c.c.), was slowly added at 90° with continuous stirring to water (200 c.c.), saturated with *n*-butanol (40 c.c.). The stirring was continued at this temperature for 2½ hours. The paste was cooled to 70° and centrifuged (10,000 r.p.m.) for 5 minutes. The temperature of the paste was readjusted to 90°. The solution was transferred to a Dewar flask and allowed to cool very slowly. After 60 hours, the amylose–butanol complex was removed in a centrifuge (5000 r.p.m.) and washed with several portions of water saturated with butanol. The precipitate was dehydrated and freed from

TABLE I.

Conditions of dispersion.							Amylose fraction.		Approx. total amylose in precipitated fraction, %	Amylopectin fraction.		Approx. total amylopectin in non-precipitated fraction, %
Conc. of starch.	Temp.	Time, hrs.	Solvent.	Starch dissolved, %.	Method.	Precipitant.	B.V.	Amylose, %.		B.V.	Amylose, %.	
3	100°	½	Water (cont. NaCl)	58	I*a*	Thymol	0·80	60 †	32	0·150	8·0 †	75
3	100	½	,,	98	I*b*	,,	0·91	66	63	0·141	7·4	89
3	100	½ *	,,	96	I*c*	,,	1·03	74	56	0·141	7·4	94
3	100	3	,,	96	I*d*	,,	1·03	74	72	0·128	6·6	92
2	90	2½	Water satd. with butanol	96	II	Butanol	0·90	65	68	0·137	7·1	87
2	90	2½	Water containing pyridine (15%)	96	III	Pyridine	0·99	71 †	76	0·111	5·3	89

TABLE I*a*.

2	90	½ *	Water cont. pyridine (20%) satd. with butanol	94	IV*a*	Pyridine–butanol	0·41	29	85	0·087	2·8 †	52
2	90	1 *	,,	96	IV*b*	,,	0·42	30	89	0·067	2·0 †	51
2	90	3 *	,,	98	IV*c*	,,	0·43	31	91	0·067	2·0	50

* 5 Minutes' high-speed stirring in an "Ato-Mix" disperser was given in addition to the time stated.

† The amylose content was determined by potentiometric titration. All other amylose contents were calculated from the blue values.

butanol by being repeatedly triturated with alcohol and then ether. The amylopectin fraction was precipitated with alcohol in the usual way.

Method III. By pyridine. The procedure of method II was followed, but pyridine (15% by wt.) was used. The complex was washed with water saturated with butanol as above.

Results are recorded in Table I.

Reprecipitation of the "amylose" fraction from methods I(*c*) and I(*d*) with thymol failed to increase the purity. Reprecipitation of the "amylose" fraction (B.V., 0·90), prepared by method II, with butanol gave a product (B.V., 1·06) richer in amylose.

Purification of the Amylose.—As indicated in Table I separation by method III using pyridine produced the "amylose" fraction containing the greatest proportion of the amylose present in the starch (76%). A larger quantity (40 g.) was, therefore, fractionated by this method. A small quantity of the amylose–pyridine complex was broken down by trituration with alcohol for analysis, from which it was calculated that the complex contained 9·0—9·5 g. of crude amylose (B.V., 0·99). The remainder was washed with water saturated with butanol and subjected to reprecipitation with butanol from 0·2% solution. Several reprecipitations were carried out (Table).

No. of reprecipitation	0	1	2	3	4	5	6	7*
Uptake of iodine detd. by potentiometric titration, %	15·3	17·1	18·0	19·2	20·1	20·8	20·9	20·9
Blue value	0·99	1·09	1·15	1·20	1·25	1·29	1·30	1·30

* This reprecipitation was carried out at an amylose concentration of 0·1%. After this reprecipitation the complex was stored in water saturated with butanol. It was calculated that the complex contained 6 g. of amylose (representing approx. 80% of the total amylose present in the starch).

Examination of the Amylose–butanol Complex.—After the second reprecipitation (Table II) the complex was examined under the microscope and appeared similar to the maize amylose–butanol complex (Schoch, *loc. cit.*), taking the form of six-segmented particles (approx. diameter 5—15 μ.). After the third reprecipitation, the complex appeared in the form of rectangular platelets and showed similarity to the maize amylose–butanol compound found by Kerr and Severson (*J. Amer. Chem. Soc.*, 1943, **65**, 193). After the fourth reprecipitation the complex appeared in the form of short thin needles similar to the potato and tapioca amylose–butanol complexes described by Kerr (*J. Amer. Chem. Soc.*, 1945, **67**, 2268; "The Chemistry and Industry of Starch, New York 1944, p. 147). The complex retained this structure after 4 more reprecipitations.

Investigation of the Amylose.—The butanol–amylose complex dissolved easily in warm water to give a clear solution which was coloured an intense blue on the addition of iodine solution (0·1N.). After 2—3 days, the amylose solution became cloudy and after 7 days a precipitate could be separated. Hydrolysis of the amylose with boiling sulphuric acid (2%) gave 98% of the theoretical yield of glucose. The complex had $[\alpha]_D^{18}$ +205° (*c*, 0·5 in water), +149° (*c*, 0·5 in N-sodium hydroxide), and +200° (*c*, 0·5 in 30% perchloric acid). (When preparing specimens for analysis, the usual method of precipitation from aqueous solution by alcohol was not adopted. An aliquot of the solution was evaporated to dryness and the weight of amylose determined directly.)

Methylation of Amylose.—The amylose (4·5 g.) was methylated under conditions similar to those used by Hirst, Jones, and Roudier (*J.*, 1948, 1779), 14 treatments being given. The product (3·8 g.) was isolated and purified by washing it with boiling water until free from sulphate and by precipitation from chloroform solution with light petroleum (b. p. 40—60°) (Found: OMe, 44·7%). The methylated amylose was obtained as a white solid forming rubber-like films which could not be powdered.

Fractionation of Methylated Amylose.—Fractionation from chloroform solution by the addition of light petroleum (b. p. 40—60°) gave: (*a*) 2·7 g. (Found: OMe, 44·8%), $[\alpha]_D^{20}$ +206° (*c*, 0·5 in chloroform), η_{sp}^{20}/c, 0·45 (*c*, 0·4 in *m*-cresol); (*b*) 0·8 g. (Found: OMe, 44·9%), $[\alpha]_D^{20}$ +204° (*c*, 0·5 in chloroform), η_{sp}^{20}/c, 0·335 (*c*, 0·4 in *m*-cresol); and (*c*) 0·2 g. (Found: OMe, 21·7%). Fraction (*c*) was not investigated further.

By use of Staudinger's equation $\eta_{sp} = Km.Mc$ (c = the concentration in g.-mol. of repeating units per l.) and $Km = 12 \times 10^{-4}$ (the value for the structurally similar methylated cellulose; Staudinger and Reinecke, *Annalen*, 1938, **535**, 47) the apparent values for the molecular weights of fractions (*a*) and (*b*) are found to 76,000 (D.P. 375) and 57,000 (D.P. 280) respectively, but since the true value for the constant is unknown these figures are only an indication of the order of magnitude of the molecular weights.

Hydrolysis of Methylated Amylose and Separation of Methylated Glucoses.—Fraction (*a*) (2·65 g.) was boiled under reflux in methanolic hydrogen chloride (150 c.c.; 1%) for 7 hours. After neutralisation with silver carbonate, treatment with hydrogen sulphide, etc., and concentration, a clear golden non-reducing syrup (2·93 g.) was obtained. The syrup was boiled under reflux with hydrochloric acid (100 c.c.; 2%) for 7 hours. After neutralisation with silver carbonate and concentration, a syrup (2·63 g.) was obtained which partly crystallised on storage. Examination of the syrup by paper chromatography (Hirst, Hough, and Jones, *loc. cit.*) indicated the presence of a single substance corresponding to 2 : 3 : 6-trimethyl glucose.

The hydrolysate was then separated on a column of cellulose (powdered Whatman No. 1 ashless filter tablets; 50 × 3·5 cm.) in the usual way (Hough, Jones, and Wadman, *loc. cit.*; Chanda *et al.*, *J.*, 1950, 1289). By elution with 50% light petroleum (b. p. 100—120°)–50% *n*-butanol, saturated with water, fractions (1) (22 mg.) and (2) (2·217 g.) were obtained, and by elution with water fraction (3) (39 mg.) was isolated (total recovery, 86%). [Groups of 5 tubes were combined and examined by paper chromatography before fraction (1) was detected.] The collected fractions were dissolved in water, warmed with charcoal, filtered through "Filter-Cel" and, after evaporation of the solvent, dried over phosphoric oxide at 35° in a vacuum.

Examination of the Fractions.—The R_G value of fraction (1) on a paper chromatogram was identical with that of tetramethyl glucopyranose. The fraction was dissolved in water (12·5 c.c.), and a portion

(2·5 c.c.) hydrolysed by boiling with sulphuric acid (1 ml.; 2%) for 6 hours. After neutralisation with barium carbonate and examination by paper chromatography, the presence of an additional substance was indicated, the R_G value of which corresponded to that of 2 : 3 : 6-trimethyl glucose. Quantitative determination of the mixture by the method of Hirst, Hough, and Jones (*loc. cit.*) [the more convenient phosphate buffer (Chanda *et al.*, *loc. cit.*) was used in this and other similar analyses] revealed the presence of 33·7% of tetramethyl glucose. Two portions (each 5 c.c.) of the solution were oxidised with buffered hypoiodite:

Titre of thiosulphate (c.c.) (0·0105N.)	2·062	2·159
Wt. of sugar oxidised (mg.)	2·56	2·68

From these results, the amount of tetramethyl glucose in fraction (1) was calculated to be 6·55 ± 0·15 mg., corresponding to the presence of one non-reducing terminal group per 400 ± 40 glucose residues. The solutions obtained after determination of the tetramethyl glucose were combined and neutralised with barium carbonate. After filtration and evaporation to dryness (reduced pressure) the residue was extracted several times with chloroform. The chloroform extract was evaporated to dryness and the residue hydrolysed with boiling sulphuric acid (1 c.c.; 2%) for 6 hours. After neutralisation paper chromatography showed the presence of 2 : 3 : 6-trimethyl glucose only (Found: 13 mg. by hypoiodite oxidation).

Fraction (2) crystallised completely and after two recrystallisations from dry ether, had m. p. 115—117° (alone or admixed with an authentic sample of 2 : 3 : 6-trimethyl glucose). The R_G value on a paper chromatogram was identical with that of 2 : 3 : 6-trimethyl glucose. The substance had $[\alpha]_D^{18}$ +94° (initial), +71° (constant) (*c*, 1·0 in water), and +69° (initial), −34° (7 hours, constant) (*c*, 1·0 in cold 2% methanolic hydrogen chloride solution) (cf. Irvine and Hirst, *loc. cit.*) (Found: OMe, 41·4. Calc. for $C_9H_{18}O_6$: OMe, 41·9%).

Fraction (3) did not crystallise; it had $[\alpha]_D^{18}$ +62° (*c*, 0·5 in water), $[\alpha]_D^{18}$ +60° (initial), −10° (6 hours const.) (*c*, 0·4 in 2% methanolic hydrogen chloride) (Found: OMe, 28·9. Calc. for $C_8H_{16}O_6$: OMe, 29·8%). Examination on the paper chromatogram showed two distinct spots corresponding to (1) 2 : 3-dimethyl glucose (R_G, 0·57) and (2) another dimethyl glucose (R_G, 0·51) (cf. Bell; Hough, Hirst, and Jones; Barker, Bourne, and Wilkinson, *locc. cit.*), the latter being in greater concentration. Traces of monomethyl glucose and glucose were also observed. Quantitative determination of the mixture of dimethyl sugars by the method of Hirst, Hough, and Jones (*loc. cit.*) indicated the presence of 2·6% of 2 : 3-dimethyl glucose (1 mg.). Fraction (3) (35 mg.) was converted into the methyl glucosides and treated with sodium metaperiodate by Bell's method (*loc. cit.*). The uptake of periodate was 0·84 mole per $C_9H_{18}O_6$ unit, indicating that fraction (3) contained 84% of 2 : 6-dimethyl glucose. After the determination, the excess of periodate was destroyed by shaking it with ethylene glycol. The glucosides were extracted, hydrolysed, and examined by paper chromatography as described for fraction (1). Two spots were observed—one (R_G, 0·57) corresponded to 2 : 3-dimethyl glucose whilst the other (R_G, 0·51) corresponded to 3 : 6-dimethyl glucose.

Partial Demethylation of 2 : 3 : 4 : 6-*Tetramethyl and* 2 : 3 : 6-*Trimethyl Glucose* (cf. Bell, *loc. cit.*).—Pure 2 : 3 : 4 : 6-tetramethyl and 2 : 3 : 6-trimethyl glucose (50 mg.) were heated in sealed tubes with 1% methanolic hydrogen chloride (1 c.c.) for 7 hours at 100°. After cooling, the acid was neutralised with silver carbonate. After filtration, the solutions were evaporated to dryness, the residues hydrolysed with 1% hydrochloric acid (2·5 c.c.) by heating at 100° for 7 hours, and the mixtures of free sugars after removal of inorganic ions were isolated as syrup which were examined by paper chromatography. The tetramethyl glucose was accompanied by trimethyl hexose (R_G, 0·82) (1%). Faint traces of dimethyl and monomethyl glucoses were also observed. The trimethyl glucose was accompanied by two dimethyl hexose spots (R_G, 0·58, 0·52) (the latter in greater concentration). A trace of monomethyl glucose was also observed. Quantitative determination of the two dimethyl sugars together indicated that they were present to an extent of 0·7%.

Determination of End Group in Barley Amylose by Periodate Oxidation.—Barley amylose (0·25 g.) was treated with sodium metaperiodate and potassium chloride (Brown, Halsall, Hirst, and Jones, *loc. cit.*), and the acid titre after 150 hours' oxidation corresponded to 0·0154 g.-mol. of formic acid from 162 g. of amylose, *i.e.*, of one mole per 65 glucose units. A similar experiment yielded one mole of formic acid per 78 glucose units. These results indicate the presence of one non-reducing terminal group per 216 ± 20 glucose residues.

Determination of the Glucose Residues linked through $C_{(1)}$, $C_{(4)}$, *and* $C_{(6)}$.—After completion of the formic acid determination (see above), ethylene glycol (1·5 c.c.) was added to the remainder to destroy excess of periodate (overnight shaking). The oxidised amylose (which had been rendered insoluble) was then dialysed until free from oxidant (test with potassium iodide and sulphuric acid, and diphenylamine and sulphuric acid) and then treated with sodium metaperiodate (15 c.c.; 0·3M.) to ensure complete oxidation. After removal of the periodate as above, the solution was evaporated to dryness and the residue hydrolysed with sulphuric acid (50 c.c.; 0·5N.) at 95° for 10 hours. After neutralisation with barium carbonate, the solution was evaporated to dryness. Only a very small white residue remained. This was dissolved in water (0·1 c.c.) and examined by paper chromatography. No glucose or other sugar was found.

Estimation of Degree of Polymerisation of Amylose, from the Reducing Power.—The method of Meyer, Noelting, and Bernfeld (*loc. cit.*) was used. A standard curve was constructed for maltose (Meyer, *loc. cit.*) by treatment of known amounts of the sugar (0·1—2 mg.) in water (3 c.c.) with 3 : 5-dinitrosalicylic acid (1 c.c.; 1·5%) and sodium hydroxide (1 c.c.; 6N.) at 65° for 30 minutes, cooling, and

diluting to 25 c.c. The solution thus obtained was compared with a blank in a " Spekker " photo-electric absorptiometer, 4-cm. cells and green filters (No. 604) being used (see Table).

Maltose, mg./25 c.c.	Molar concn. per l. ($\times 10^4$).	" Spekker " drum readings, log I/I_0.	Maltose, mg./25 c.c.	Molar concn. per l. ($\times 10^4$).	" Spekker " drum readings, log I/I_0.
0·2	0·23	0·009	1·2	1·40	0·315
0·5	0·58	0·067	1·5	1·75	0·524
0·75	0·88	0·138	1·8	2·11	0·745
1·0	1·17	0·241	2·0	2·34	0·970

For determination of the degree of polymerisation of amylose, two equal samples (50—60 mg.) were dissolved in water (3 c.c.) containing sodium hydroxide (1 c.c.; 6N.). Water (1 c.c.) was added to one sample (A, the blank) and 3 : 5 dinitrosalicylic (1 c.c.; 1·5%) to the other (B). The samples were heated as before and cooled. 1 C.c. of the dinitrosalicylic acid solution was added to A and both were made up as before. Log I/I_0 varied between 0·112 and 0·223, corresponding to a degree of polymerisation of 135 $\pm$ 20 glucose units.

" Amylopectin " fraction.

The amylopectin fractions obtained from the various precipitation procedures (Table I) were dispersed as before and treated with the various precipitants. In no case was there any significant precipitation.

Separation of " Amylopectin " with Low Amylose Content.—The method of Higginbotham and Morrison (*loc. cit.*) was used. Dry starch (5 g.), suspended in water (20 c.c.), was added with stirring to water (180 c.c.) at 90° containing pyridine (50 c.c.). The paste was stirred for various periods of time (Table Ia) and then subjected to 5 minutes' high-speed stirring in an " Ato-Mix " disperser. The paste was cooled to 70° and centrifuged (10,000 r.p.m.) for 5 minutes. The temperature of the paste was adjusted to 90° and sufficient butanol was added to saturate the solution. After transference to a Dewar flask, the solution was allowed to cool very slowly and the fractions separated as before (Table Ia).

A further portion (20 g.) was fractionated by this method (1 hour's stirring), to give 11·1 g. of amylopectin (B.V. 0·067) containing 2% of amylose. The white solid dispersed readily in warm water on stirring. The solution gave a blue colour with iodine (0·1N.). The addition of further iodine yielded a reddish-purple colour. The material had $[\alpha]_D^{15}$ +168° (*c*, 0·76 in 30% perchloric acid) and +149° (*c*, 0·75 in N-sodium hydroxide).

Methylation of Amylopectin.—Amylopectin (9·1 g.) was methylated as was the amylose fraction. After 6 treatments with the reagents, the product was purified as before (Found : OMe, 44·0%) (yield, 7·3 g.).

Fractionation from chloroform solution by the addition of light petroleum (b. p. 40—60°) gave (*a*) 5·6 g., $[\alpha]_D^{17}$ +204° (*c*, 0·5 in chloroform) (Found : OMe, 44·0%), (*b*) 1·5 g., $[\alpha]_D^{17}$ +204° (*c*, 0·5 in chloroform) (Found : OMe, 43·9%), and (*c*) 0·2 g. (Found : OMe, nil).

Fraction (*a*) had η_{sp}/c 3·2 (*c*, 0·4 in *m*-cresol) corresponding to an apparent M = 485,000 (see Hirst and Young, *J.*, 1939, 1475).

Fraction (*b*) had η_{sp}/c 1·5 (*c*, 0·4 in *m*-cresol), corresponding to an apparent M = 215,000.

Fraction (*a*) (4·3 g.) was treated four times in boiling methyl iodide (100 g.) with dry silver oxide (25 g.), added in small portions during 24 hours. After filtration and extraction with hot chloroform fraction (a_1) (3·6 g.) was obtained (Found : OMe, 45·0%). The product was essentially homogeneous and had a molecular weight similar to that before treatment.

Hydrolysis of Methylated Amylopectin and Separation of Methylated Glucoses.—(a) *By paper chromatography.* Fraction (a_1) (50 mg.) was hydrolysed in a sealed tube by the method of Hough, Hirst, and Jones (*loc. cit.*). Analysis by paper chromatography revealed the following :

Sugar indicated.	R_G.	Wt., %.
2 : 3 : 4 : 6-Tetramethyl glucose	1·0	4·2
2 : 3 : 6-Trimethyl glucose	0·80	90·5
(?2 : 3-)Dimethyl glucose	0·55	5·3

Traces of monomethyl glucose and glucose were also observed. These results indicate the presence of one non-reducing terminal group per 25 glucose residues.

(b) *By a cellulose column.* Fraction (a_1) (3·22 g.) was hydrolysed as described for the methylated amylose. The mixture of methylated glucoses (3·25 g., 92%) was separated on a column of cellulose (80 × 3·3 cm.) as before. By elution with 50% light petroleum (b. p. 100—120°)–50% *n*-butanol, saturated with water, fractions (1) (152 mg.), (2) (2·679 g.), (3) (140 mg.), and (4) (32 mg.) were isolated. By elution with water, a small fraction (less than 10 mg.) was obtained; paper chromatography indicated that this fraction, which was not investigated further, contained a monomethyl glucose. The total recovery was 93%.

Examination of the Fractions.—Fraction (1). Paper chromatography revealed the presence of a single spot (R_G, 1·0) corresponding to tetramethyl glucose. Hypoiodite oxidation revealed that the fraction was 84·9% pure. A portion (10 mg.) was hydrolysed with boiling sulphuric acid (1 c.c.; 2%) for 6 hours. Re-examination by paper chromatography showed the presence of an additional substance

(R_G, 0·81) corresponding to 2 : 3 : 6-trimethyl glucose. Fraction (1) (134 mg.) was therefore hydrolysed with hydrochloric acid (10 c.c.; 1%) for 7 hours. The hydrolysate was separated on a cellulose column (35 × 1·3 cm.) as before, to give fractions (1*a*) (106 mg.) and (1*b*) (21 mg.) (recovery, 95%). Fraction (1*a*) partly crystallised on storage. After two recrystallisations from light petroleum (b. p. 40—60°) it had m. p. 85—87° (not depressed on admixture with tetramethyl D-glucopyranose), $[\alpha]_D^{19}$ +84° (*c*, 0·45 in water) (Found : OMe, 52·1. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%). The anilide had m. p. 137—138° (alone or admixed with authentic tetramethyl D-glucopyranose anilide) (Found : N, 4·4; OMe, 39·5. Calc. for $C_{16}H_{25}O_5N$: N, 4·5; OMe, 39·9%). From the above results the amount of tetramethyl glucose was calculated to be 118 ± 12 mg., corresponding to one non-reducing terminal group per 26 ± 2 glucose residues.

Fraction (1*b*) did not crystallise. Paper chromatography indicated the presence of a single substance (R_G, 0·81) corresponding to 2 : 3 : 6-trimethyl glucose, $[\alpha]_D^{19}$ +68·4° (*c*, 0·4 in water), $[\alpha]_D^{19}$ +67·1° (initial), −36·9° (7 hours, constant) (*c*, 0·4 in cold dry 2% methanolic hydrogen chloride) (Found : OMe, 40·8. Calc. for $C_9H_{18}O_6$: OMe, 41·9%).

Fraction (2) crystallised partly when kept. The material was recrystallised twice from dry ether (50% yield) and had m. p. 115—117° alone or admixed with an authentic sample of 2 : 3 : 6-trimethyl D-glucose. The R_G value on a paper chromatogram was identical with that of 2 : 3 : 6-trimethyl glucose. The crystalline material had $[\alpha]_D^{19}$ +90° (initial), 71° (constant) (*c*, 1 in water), and $[\alpha]_D^{19}$ +68·4° (initial), −35·0° (7 hours, constant) (*c*, 1·0 in cold 2% methanolic hydrogen chloride solution) (Found : OMe, 41·6. Calc. for $C_9H_{18}O_6$: OMe, 41·9%).

Fraction (3) was obtained as a pale yellow syrup which partly crystallised during 2 weeks in the cold. Recrystallised once from dry ethyl acetate it had m. p. 84—86°. The R_G value (0·57) on a paper chromatogram was identical with that of 2 : 3-dimethyl glucose. The crystalline material had $[\alpha]_D^{19}$ +113° (initial), +65° (final) (*c*, 1·0 in water) (Found : OMe, 29·4. Calc. for $C_8H_{16}O_6$: OMe, 29·7%). The 2 : 3-dimethyl gluconophenylhydrazide (Evans, Levi, Hawkins, and Hibbert, *Canad. J. Res.*, 1942, **20**, *B*, 175) had m. p. 160—162° (Evans *et al.*, 166·5—167°) (Found : C, 53·7; H, 7·1; N, 9·0; OMe, 19·3. Calc. for $C_{14}H_{22}O_6N_2$: C, 53·5; H, 7·0; N, 8·9; OMe, 19·7%).

Fraction (4) was obtained as a syrup which failed to crystallise during several weeks in the cold. It had $[\alpha]_D^{19}$ +78° (initial), +62° (final) (*c*, 1·0 in water), $[\alpha]_D^{19}$ +61° (initial), −11° (final, after 8 hours) (*c*, 0·5 in cold 2% methanolic hydrogen chloride solution) (Found : OMe, 28·9. Calc. for $C_8H_{16}O_6$: OMe, 29·7%). The fraction (25 mg.) was converted into the methylglucosides and treated with sodium metaperiodate by Bell's method (*loc. cit.*). The uptake of sodium metaperiodate was 79% per $C_8H_{16}O_6$ unit, indicating that the fraction contained 79% of 2 : 6-dimethyl glucose.

Determination of End Group in Amylopectin and in Whole Starch by Periodate Oxidation.—(a) *Amylopectin.* This was carried out by the method described above for amylose. The yield of formic acid from 162 g. of amylopectin after 150 hours' oxidation was 0·0412 g.-mol., corresponding to one non-reducing terminal group per 24 glucose residues.

(b) *Barley starch.* The yield of formic acid from 162 g. of starch after 150 hours' oxidation was 0·033 g.-mol., corresponding to one non-reducing terminal group per 30 glucose residues. If the amount of formic acid yielded from the amylose fraction is neglected then the formic acid yield from the whole starch corresponds to the presence of one non-reducing terminal group per 24 glucose residues in the amylopectin fraction.

Determination of Glucose Residues in Amylopectin linked through $C_{(3)}$, $C_{(4)}$, *and* $C_{(6)}$.—This was carried out by the method described by Hirst, Jones, and Roudier (*loc. cit.*) on (*a*) the amylopectin fraction and (*b*) the whole starch. On hydrolysis and examination by paper chromatography, glucose was found in each case. Determination by the method of Flood, Hirst, and Jones (*J.*, 1948, 1679) using xylose as standard gave : for (*a*), 0·59 g. of glucose from 100 g. of amylopectin, and (*b*) 1·8 g. of glucose from 100 g. of starch. These results indicate that (i) over 86% of the linkages in the amylopectin fraction are 1 : 6-linkages, and (ii) that about 50% of the linkages in the amylopectin fraction are 1 : 6-linkages. It is thought probable that the closely packed granular structure prevented complete oxidation by the periodate in case (*b*).

Attempted Estimation of Degree of Polymerisation of Amylopectin.—The method used by Meyer *et al.* (*loc. cit.*) gave very low "Spekker" readings with separated amylopectin and with whole starch. The results indicated that the molecular weights were in excess of 240,000.

Thanks are expressed to the Institute of Brewing Research Foundation under whose auspices this work was carried out, and to Professor E. L. Hirst, F.R.S., for his interest. We are also indebted to Messrs. William Younger and Co. Ltd. for the gift of barley and to Professor I. A. Preece for the details of the method for separation of the starch.

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500. *Studies on Phosphorylation. Part VIII.* The Production of Tetrabenzyl Pyrophosphate by the Action of Acyl Chlorides on Dibenzyl Hydrogen Phosphate, and a Novel Reaction of Tetraphenyl Pyrophosphate.*

By H. S. Mason and A. R. Todd.

The action of thionyl chloride and other acyl halides on dibenzyl hydrogen phosphate yields tetrabenzyl pyrophosphate rather than dibenzyl chlorophosphonate. With oxalyl chloride the initial product is a mixed anhydride, bisdibenzylphosphoryl oxalate, which yields carbon monoxide, carbon dioxide, and tetrabenzyl pyrophosphate when heated. Tetraphenyl pyrophosphate has been prepared and shown to be a powerful phosphorylating agent. It reacts very readily with dibenzyl hydrogen phosphate in presence of bases, yielding tetrabenzyl pyrophosphate and diphenyl hydrogen phosphate. The implications of these various observations in the general field of nucleotide synthesis are discussed.

The researches which form the subject of this series of papers have as their primary object the development of satisfactory methods for the phosphorylation and polyphosphorylation of nucleosides so as to provide an adequate basis for synthetic work in the fields of nucleotide, nucleotide coenzyme, and polynucleotide chemistry which have been a major topic of interest in these laboratories in recent years. They are indeed an essential part of our nucleotide studies. Broadly speaking, the problem of nucleotide synthesis resolves itself into two parts: (*a*) the phosphorylation and polyphosphorylation of nucleosides and related compounds to yield mono-esters of the oxyacids of phosphorus, *e.g.*, simple monoribonucleotides, adenosine diphosphate, adenosine triphosphate; and (*b*) the linkage of two dissimilar nucleoside molecules through phosphate or pyrophosphate groupings, *i.e.*, the synthesis of unsymmetrical diesters of phosphoric acid, RO·P(O)(OH)·OR′, or pyrophosphoric acid, R·P(O)(OH)·O·P(O)(OH)·OR′, to which types belong the polynucleotides and a number of nucleotide coenzymes such as cozymase. Earlier papers of this series (*J.*, 1945, 382, 660; 1947, 674; 1948, 1106; 1949, 815; 1950, 2023, 2030) have been concerned mainly with part (*a*), and methods have been devised whereby mono-esters of phosphoric and polyphosphoric acids may be prepared. These methods have been applied successfully to the synthesis of various mononucleotides (Baddiley and Todd, *J.*, 1947, 648; Michelson and Todd, *J.*, 1949, 2476; Brown, Haynes, and Todd, *J.*, 1950, 3299), of adenosine diphosphate (Baddiley and Todd, *loc. cit.*), and of adenosine triphosphate (Baddiley, Michelson, and Todd, *J.*, 1949, 582; Michelson and Todd, *ibid.*, p. 2487). The present paper records some of our results in initial approaches to the solution of the second main problem of nucleotide synthesis described under (*b*) above.

The use of a bifunctional phosphorylating agent such as phenyl dichlorophosphinate † ($PhO{\cdot}POCl_2$) offers a fairly obvious route to symmetrical diesters of phosphoric acid and, indeed, Gulland and Smith (*J.*, 1948, 1532) have used it to prepare a diuridine phosphate; since the latter was prepared from benzylideneuridine, it should now be regarded as di(uridine-5′) hydrogen phosphate (Brown, Haynes, and Todd, *loc. cit.*). The reaction of phenyl dichlorophosphinate with one equivalent of an alcohol to give an alkyl phenyl chlorophosphonate, PhO·P(O)(OR)Cl, which can react further with a second alcohol, indicates the possible application of this reagent to the preparation of unsymmetrical diesters. An example of its use in this way is to be found in the preparation of "α-L-glycerylphosphorylcholine" by Baer and Kates (*J. Amer. Chem. Soc.*, 1948, 70, 1394). For our purposes, this route, although under investigation, is likely to be of limited value since the presence of a phenyl group in the initial product of phosphorylation would be a disadvantage in so far as its removal might be impossible without disruption of a sensitive molecule. Clearly, the simplest solution would be to convert a nucleoside benzyl hydrogen phosphate, which is readily accessible, into a phosphorylating agent by replacement of the acidic hydroxy-group by halogen or some similar grouping and use the product to phosphorylate a second nucleoside. Model experiments have been carried out with this end in view. Another method which might have a limited application would be the

* Part VII, *J.*, 1950, 2030.

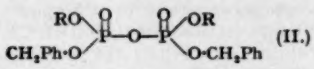
† For the nomenclature now used, see *J.*, 1951, 1868, footnote.

reaction of a nucleoside benzyl hydrogen phosphate with a nucleoside derivative containing an epoxide grouping; experiments bearing on this route will be described in a separate communication.

Deutsch and Fernö (*Nature*, **1945**, **156**, 604) stated that reaction of thionyl chloride with potassium dibenzyl phosphate yields dibenzyl chlorophosphonate, although no details were given. Some time ago, Mr. J. J. Michalski in these laboratories tried to repeat this work, assaying the amount of chlorophosphonate produced by reaction of the product with ammonia and weighing the dibenzyl aminophosphonate produced. In no case did his yield of product exceed 50% of the theoretical, and dibenzyl hydrogen phosphate was always obtained in addition to the aminophosphonate. Replacement of the thionyl chloride in this reaction by phosphorus pentachloride or carbonyl chloride gave results similar in nature but much inferior. His tentative assumption that the reaction of thionyl chloride with potassium dibenzyl phosphate yields a pyrophosphate rather than a chlorophosphonate has been found valid. Tetrabenzyl pyrophosphate has been isolated from the reaction product and no dibenzyl chlorophosphonate was detected. A series of experiments was carried out using thionyl chloride alone and in presence of various tertiary bases, in various hydrocarbon solvents, and employing dibenzyl hydrogen phosphate free or as its sodium, potassium, or silver salt; in no case was clear evidence for the formation of chlorophosphonate obtained. That the product obtained from such reactions should be tetrabenzyl pyrophosphate rather than dibenzyl chlorophosphonate was what one would expect, since the standard methods for the preparation of pyrophosphates include reaction of dialkyl chlorophosphonates with metallic salts of dialkyl hydrogen phosphates (cf., *e.g.*, Baddiley and Todd, *loc. cit.*) or with the free acids in presence of tertiary bases (Toy, *J. Amer. Chem. Soc.*, **1950**, **72**, 2065).

Oxalyl chloride has been employed for the conversion of acids into acyl chlorides. It was found to react readily with dibenzyl hydrogen phosphate; when a mixture of the two compounds was warmed hydrogen chloride was evolved and a crystalline product was obtained which on the basis of analysis and chemical behaviour is regarded as the mixed anhydride bisdibenzylphosphoryl oxalate (I). The anhydride (I) when heated to its melting point evolved

(I.) $(CH_2Ph\cdot O)_2P(=O)\cdot O\cdot CO\cdot CO\cdot O\cdot P(=O)(O\cdot CH_2Ph)_2$



(II.)

carbon monoxide and carbon dioxide, yielding a product which was evidently tetrabenzyl pyrophosphate since it furnished dibenzyl aminophosphonate and dibenzyl hydrogen phosphate on treatment with ammonia. An exactly similar decomposition of the anhydride was effected by treatment with a tertiary base; direct treatment with ammonia yielded oxamide, dibenzyl aminophosphonate, and dibenzyl hydrogen phosphate. Oxalyl chloride appeared to react in the same way with diphenyl hydrogen phosphate. In this instance, the mixed anhydride could not be isolated because of its great sensitivity to moisture, but the reaction product effervesced when heated above 100°, and from the resulting oil diphenyl aminophosphonate could be obtained in fair yield by treatment with ammonia.

The formation of pyrophosphates in the experiments above described is not necessarily a serious drawback from the standpoint of developing methods for the synthesis of unsymmetrical diesters of phosphoric and polyphosphoric acids. Tetraesters of pyrophosphoric acid, being acid anhydrides, can be used as phosphorylating agents; tetrabenzyl pyrophosphate, for example, will phosphorylate alcohols readily in the presence of tertiary bases (Atherton and Todd, *J.*, 1947, 674). The readiness with which such esters act as phosphorylating agents depends *inter alia* on the nature of the ester groups, or, more precisely, on the acid strength of the diesters of phosphoric acid from which they are derived. Thus tetraphenyl pyrophosphate, which can readily be prepared from silver diphenyl phosphate and diphenyl chlorophosphonate, decomposes rapidly in moist air, and it will phosphorylate alcohols even in the absence of bases; the preparation of *p*-nitrobenzyl diphenyl phosphate by this means is described in the Experimental portion. One method, although perhaps an uneconomic one, of achieving our desired aim would be to convert a nucleotide derivative such as benzyl 2′ : 3′-*iso*propylidene adenosine-5′ hydrogen phosphate into its anhydride, dibenzyl di-(2′ : 3′-*iso*propylidene adenosine-5′) pyrophosphate (II; R = 2′ : 3′-*iso*propylidene adenosine-5′) and bring this substance into reaction with a second nucleoside in presence of a tertiary base; protecting benzyl groups could subsequently be removed by hydrogenation or hydrolysis. Since reaction with a nucleoside would produce not merely the required dinucleotide but also

an equivalent amount of the original benzyl 2′ : 3′-*iso*propylidene adenosine-5′ hydrogen phosphate, the method might well prove rather cumbersome in operation. Nevertheless, some unsuccessful attempts were made to prepare the pyrophosphate (II; R = 2′ : 3′-*iso*propylidene adenosine-5′) from benzyl 2′ : 3′-*iso*propylidene adenosine-5′ hydrogen phosphate or its salts by reaction with thionyl chloride. The silver salt reacted readily with thionyl chloride to produce a metastable soluble complex containing silver, sulphur, and chlorine whose precise structure was not determined. The complex decomposed when heated, with production of silver chloride and sulphur dioxide, but we were unable to isolate any pyrophosphate or chlorophosphonate from the resulting complex mixture of organic materials. The pyrophosphate could not be prepared *via* a mixed anhydride with oxalic acid since benzyl 2′ : 3′-*iso*propylidene adenosine-5′ hydrogen phosphate as well as adenosine and adenine itself reacted with oxalyl chloride to produce stable, bright yellow products from which the original purine derivatives could not be easily regenerated.

A new method for the preparation of certain tetraesters of pyrophosphoric acid has been found during experiments with tetraphenyl pyrophosphate. In the presence of a tertiary base this substance reacts very rapidly with dibenzyl hydrogen phosphate yielding tetrabenzyl pyrophosphate and diphenyl hydrogen phosphate. On the evidence of a series of experiments it appears that triethylamine is a very effective agent for bringing about reaction and dimethylformamide is the preferred solvent; under these conditions the exchange reaction—presumably a two-stage nucleophilic displacement—is almost quantitative. A consideration of this reaction indicates that, appropriately modified, it might have far-reaching applications in the synthesis not only of unsymmetrical diesters of phosphoric acid but also of unsymmetrical pyrophosphates. Exploration of these possible developments and their extension to the nucleotide field are at present in progress.

Experimental.

Action of Thionyl Chloride on Dibenzyl Hydrogen Phosphate : Isolation of Tetrabenzyl Pyrophosphate.—Anhydrous dibenzyl hydrogen phosphate (1·5 g.) was dissolved in benzene (25 c.c.), and remaining traces of moisture were removed by a preliminary distillation of the azeotrope. To the boiling solution freshly distilled thionyl chloride (5 c.c.) was added, and the mixture heated under reflux for 18 hours, then evaporated to dryness under reduced pressure. The residual oil was dissolved in chloroform, and the solution washed with aqueous sodium hydrogen carbonate, dried, and evaporated. The brownish oil was dissolved in a minimum of boiling *cyclo*hexane, treated with charcoal, filtered, and set aside. Tetrabenzyl pyrophosphate separated; recrystallised from *cyclo*hexane, it had m. p. 62°, undepressed on admixture with an authentic specimen (m. p. 62°) (yield, 0·3 g.).

Bisdibenzylphosphoryl Oxalate (I).—Anhydrous dibenzyl hydrogen phosphate (1·0 g.) was dissolved in freshly distilled oxalyl chloride (0·5 c.c.), and the mixture heated to boiling for 4 minutes in absence of moisture, then evaporated to dryness at room temperature under reduced pressure (0·2 mm.), the process being continued for 3 hours to ensure complete removal of oxalyl chloride. The crystalline *anhydride* so obtained (yield, theoretical) had m. p. 107—108° (efferv.) (Found : C, 58·9; H, 4·6. $C_{28}H_{26}O_{10}P_2$ requires C, 59·0; H, 4·6%). On further heating of the molten anhydride decomposition and polymerisation occurred at 185°; the same behaviour was shown by tetrabenzyl pyrophosphate heated above its m. p.

Pyrolysis. The mixed anhydride (from 1 g. of dibenzyl hydrogen phosphate) was kept at its m. p. until effervescence ceased. The product was cooled and dissolved in liquid ammonia, and the solvent allowed to evaporate after 1 hour. The residue (0·3 g.) was washed thoroughly with water, dried, and recrystallised from aqueous ethanol; it then had m. p. 103°, undepressed on admixture with an authentic specimen (m. p. 103—104°) of dibenzyl aminophosphonate.

The presence of carbon dioxide in the gases evolved on pyrolysis was shown by reaction with barium hydroxide solution, and that of carbon monoxide by the appearance of the carbon monoxide-hæmoglobin absorption bands on passing the mixed gases through a solution of hæmoglobin. The mixed anhydride also decomposed when heated in a micro-combustion furnace with use of a cold filling which prevents conversion of carbon monoxide into carbon dioxide; 53·57 mg. treated in this way gave 1·7 mg. of carbon dioxide, *i.e.*, 44·5% of the theoretical for 1 mol. By the hot-tube technique, 48·37 mg. yielded 3·35 mg. of carbon dioxide, *i.e.*, 42% of the theoretical for 2 mols. It is thus concluded that carbon monoxide and carbon dioxide are evolved in equimolar quantities.

Action of ammonia. A solution of the mixed anhydride (0·16 g.) in a small amount of liquid ammonia was allowed to evaporate slowly overnight; some frothing was observed in the early stages of the reaction. The residue was triturated with ethanol; a white residue of oxamide (8 mg., 35%) remained which did not melt below 310°. Precipitation of the ethanolic solution with water yielded dibenzyl aminophosphonate (8 mg.), and acidification of the aqueous ethanolic mother-liquor gave dibenzyl hydrogen phosphate (80 mg.).

Action of Oxalyl Chloride on Diphenyl Hydrogen Phosphate.—Anhydrous diphenyl hydrogen phosphate (0·5 g.) was dissolved in dry chloroform (3 c.c.), freshly distilled oxalyl chloride (0·4 c.c.) added, and the

mixture refluxed for 20 minutes with exclusion of moisture. Solvent was removed at 0·2 mm., and the residue dissolved in hot *cyclo*hexane. On cooling, small rosettes of crystalline material began to deposit, but these were subsequently resorbed by an oil and could not be obtained pure. When the oily product was warmed to about 100°, gas was evolved and a portion of the resulting material (45 mg.), treated with concentrated aqueous ammonia, gave diphenyl aminophosphonate (11 mg., 50%).

Tetraphenyl Pyrophosphate.—Equimolar quantities of silver diphenyl phosphate and diphenyl chlorophosphonate were mixed together in the bulb of a molecular still (pistol type) which was rapidly evacuated, set in a vertical position, and heated in an oil-bath for 10 minutes at 125°. The bulb was then raised to the distillation position, and the bath-temperature raised to 165°, any material distilling below this being discarded. *Tetraphenyl pyrophosphate* distilled at 165° (bath-temp.)/0·1 mm. as a colourless liquid, leaving a residue of silver chloride (Found: C, 59·2; H, 4·4. $C_{24}H_{20}O_7P_2$ requires C, 59·8; H, 4·1%); it is very rapidly attacked by moist air, yielding diphenyl hydrogen phosphate, and it must be stored in sealed ampoules.

Phosphorylation with Tetraphenyl Pyrophosphate: p-*Nitrobenzyl Diphenyl Phosphate.*—A mixture of *p*-nitrobenzyl alcohol (0·2 g.), tetraphenyl pyrophosphate (0·5 g.), and dry chloroform (5 c.c.) was refluxed for 3 hours, then chloroform was removed under reduced pressure and replaced by benzene (5 c.c.). The benzene solution was set aside at 0° for 24 hours; unchanged *p*-nitrobenzyl alcohol (0·025 g.) separated and was removed by filtration. The filtrate was evaporated to dryness, the residue dissolved in ether, and the solution washed with sodium hydrogen carbonate, dried, and evaporated. The residue of p-*nitrobenzyl diphenyl phosphate*, recrystallised from ethanol, had m. p. 60—62° (0·113 g.), undepressed on admixture with a sample (m. p. 65°) prepared by Dr. C. W. Taylor from *p*-nitrobenzyl alcohol and diphenyl chlorophosphonate (Found: C, 59·4; H, 4·2. $C_{19}H_{16}O_6NP$ requires C, 59·2; H, 4·2%).

Exchange Reaction between Tetraphenyl Pyrophosphate and Dibenzyl Hydrogen Phosphate.—(1) *In presence of pyridine.* In a first experiment tetraphenyl pyrophosphate (0·14 g.) was added to a solution of dibenzyl hydrogen phosphate (0·24 g.) in pyridine (1 c.c.), and the mixture left at 0° for 1 minute. Pyridine was removed under reduced pressure at room temperature and concentrated aqueous ammonia (*d* 0·880) was added. The solid product was shown to be dibenzyl aminophosphonate by m. p. and mixed m. p. (102—104°); its yield (21 mg.) was 25·7% of theory based on formation of tetrabenzyl pyrophosphate by the exchange reaction. In a second experiment a mixture of tetraphenyl pyrophosphate (0·25 g.), dibenzyl hydrogen phosphate (0·25 g.), and pyridine (0·5 c.c.) was left for 1 minute at room temperature and worked up in the same way; the yield of dibenzyl aminophosphonate obtained was 40% on the same basis.

In a third experiment, dibenzyl hydrogen phosphate (0·5 g.) was dissolved in pyridine (1 c.c.), and tetraphenyl pyrophosphate (0·5 g.) added. After 1 minute at room temperature pyridine was removed at 0·1 mm. The residue was suspended in water (10 c.c.), and dilute ammonia was added cautiously with shaking until the mixture was weakly alkaline. Excess of ammonia was removed under reduced pressure and the suspension was set aside overnight. The colourless needles (0·12 g.; m. p. 55—60°) which separated yielded pure tetrabenzyl pyrophosphate (m. p. and mixed m. p. 60—62°) on recrystallisation from *cyclo*hexane.

(2) *In presence of triethylamine.* Tetraphenyl pyrophosphate (0·5 g.) was added to a solution of dibenzyl hydrogen phosphate (0·5 g.) and triethylamine (0·2 c.c.) in dimethylformamide (2 c.c.) at room temperature. After 5 minutes, concentrated aqueous ammonia (10 c.c.; *d* 0·880) was added with shaking, and the mixture left for 1 hour. On dilution with water, needles of dibenzyl aminophosphonate separated; recrystallised from carbon tetrachloride, the product had m. p. 102—104°, undepressed by an authentic specimen (m. p. 103—104°). The yield was 0·21 g., or 84% of theory based on tetrabenzyl pyrophosphate.

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501. *Studies on Phosphorylation. Part IX.* The Preparation of Phosphates of Carbohydrate Derivatives by the Epoxide Route.*

By W. E. Harvey, J. J. Michalski, and A. R. Todd.

The preparation of phosphate esters by addition of dibenzyl hydrogen phosphate to ethylene oxide derivatives (Part V; *J.*, 1949, 815) has been extended to epoxides of the carbohydrate series to assess the potentialities of the method for use in the field of nucleotide synthesis. 4 : 6-Benzylidene α-methyl-2 : 3-anhydro-D-allopyranoside (II) reacts with dibenzyl hydrogen phosphate much less readily than does 1 : 2-*iso*propylidene 5 : 6-anhydro-α-D-glucofuranose (I) and gives a mixture of benzylidene methylhexoside dibenzyl phosphates. Removal of the benzyl and benzylidene groups gives a mixture of α-methyl-D-altropyranoside-2 phosphate (isolated as barium salt) and α-methyl-D-glucopyranoside-3 phosphate in which the former predominates. 7-β-(4 : 6-Benzylidene 2 : 3-anhydro-D-allopyranosyl)theophylline (VIII) reacted with dibenzyl hydrogen phosphate when heated in dioxan solution; in this case the product isolated appeared to be 7-β-D-glucopyranosyltheophylline-3′ phosphate (IX).

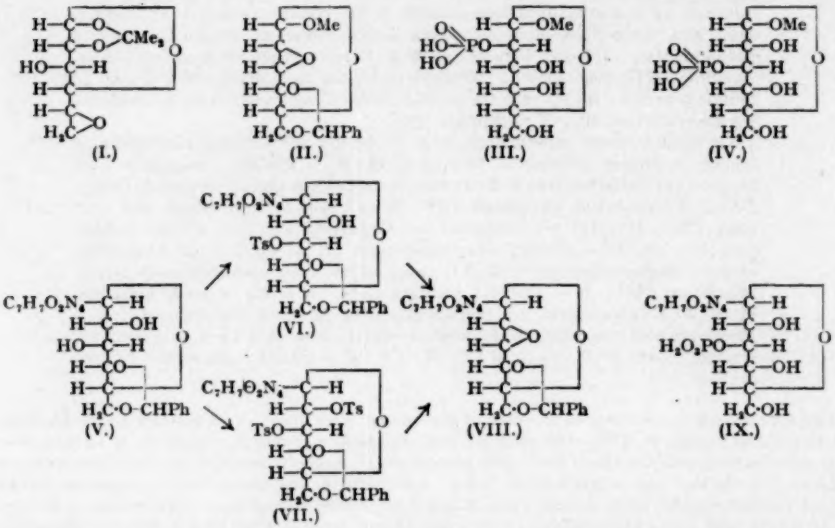
In further model experiments, 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 benzyl hydrogen phosphate (X; R = H, R′ = $PhCH_2$) condensed with *cyclo*hexene oxide to give (1 : 2 : 3 : 4-tetra-acetyl-β-D-glucopyranose-6) benzyl 2-hydroxy*cyclo*hexyl phosphate (XI; R = $PhCH_2$) from which the acid ester (XI; R = H) was obtained by hydrogenation. In similar fashion (X; R = H, R′ = $PhCH_2$) condensed with (I) to give (1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6)(1 : 2-*iso*propylidene α-D-glucofuranose-6) benzyl phosphate (XII; R = $PhCH_2$), yielding (XII; R = H) on hydrogenation. (1 : 2 : 3 : 4-Tetra-acetyl β-D-glucopyranose-6) (2 : 3 : 4 : 6-tetra-acetyl β-D-glucopyranose-1) hydrogen phosphate (XIII) has also been prepared by starting from the silver salt of (X; R = H, R′ = $PhCH_2$) and α-acetobromoglucose.

The first recorded synthesis of an ester of phosphoric acid from a 1 : 2-epoxide is due to Bailly (*Ann. Chim.*, 1916, **6**, 133), who showed that disodium α-glyceryl phosphate is formed from glycide alcohol and disodium hydrogen phosphate in aqueous solution at room temperature. Later, Zetsche and Aeschlimann (*Helv. Chim. Acta*, 1926, **9**, 708) showed that syrupy phosphoric acid reacted readily with glycide alcohol and epichlorohydrin giving, respectively, α-glyceryl phosphate and two chlorohydroxypropyl phosphates, and further that α-glyceryl phosphate reacted with epichlorohydrin to give a mixture of diesters. Eidebenz and Depner (*Arch. Pharm.*, 1942, **280**, 227) obtained either a mono- or a di-ester by varying the molar ratio of phosphoric acid which was allowed to react with epi-iodohydrin, and an American patent specification (Adams and Shoemaker, 1945; U.S.P. 2,372,244) describes the reaction of phosphoric and phosphorous acid with ethylene oxides to give phosphates and phosphites and indicates that the sulphur and nitrogen analogues of the oxides behave in a similar way. In Part I of this series (Atherton, Openshaw, and Todd, *J.*, 1945, 382) Bailly's observation was extended to the preparation of 2-hydroxyethyl phosphate and 2-hydroxypropyl phosphate by interaction of disodium hydrogen phosphate with ethylene and propylene oxides. Since then, Lampson and Lardy (*J. Biol. Chem.*, 1949, **181**, 697), apparently unaware of the earlier literature, have described the preparation of 2-hydroxypropyl phosphate from propylene oxide and dipotassium hydrogen phosphate and have also made glucose-6 phosphate by a similar method starting from 1 : 2-*iso*propylidene 5 : 6-anhydro-α-D-glucofuranose (*ibid.*, p. 693). As indicated briefly in Part I (*loc. cit.*), we were interested in the possible application of the method to carbohydrate epoxide derivatives as a possible route to the synthesis of compounds of the dinucleotide type. Baddiley, Clark, Michalski, and Todd (Part V; *J.*, 1949, 815) showed that dibenzyl and substituted dibenzyl hydrogen phosphates react with *cyclo*hexene oxide to yield the expected triesters of phosphoric acid, and it was clear that if the method could be applied to nucleoside epoxides, then not only could dibenzyl and thence monobenzyl nucleoside phosphates be

* Part VIII, preceding paper.

prepared but the latter might be brought into reaction with a second nucleoside epoxide to produce a dinucleotide. The method could not, of course, apply to nucleotides derived from the naturally occurring ribonucleosides since the latter are D-ribofuranose derivatives with a *cis*-arrangement of hydroxyls, and the opening of an epoxide would inevitably yield a *trans*-arrangement of groups, but it could be of value in other cases.

Although 1 : 2-*iso*propylidene 5 : 6-anhydro-α-D-glucofuranose (I) reacts with dipotassium or disodium hydrogen phosphate (Lampson and Lardy, *loc. cit.*), the latter salt failed to react in aqueous dioxan solution with 4 : 6-benzylidene α-methyl-2 : 3-anhydro-D-allopyranoside (II) even when heated at 150° for 36 hours. When dibenzyl hydrogen phosphate and 1 : 2-*iso*propylidene 5 : 6-anhydro-α-D-glucofuranose were heated in carbon tetrachloride solution under reflux for 24 hours, a neutral product was obtained which, although it could not be crystallised, was evidently 1 : 2-*iso*propylidene α-D-glucofuranose-6 dibenzyl phosphate, since hydrogenolysis followed by removal of the *iso*propylidene group yielded the known glucose-6 phosphate.



Monodebenzylation of the neutral ester gave an acidic material believed to be the corresponding monobenzyl ester, which, however, could not be crystallised either as the free acid or as its *cyclo*hexylamine salt. 4 : 6-Benzylidene α-methyl-2 : 3-anhydro-D-allopyranoside (II) also reacted with dibenzyl hydrogen phosphate in carbon tetrachloride solution, although much less readily than (I), 7 days' heating being required to complete the reaction. The product was a colourless neutral resin which, after chromatography on alumina, gave analytical values in good agreement with those required for a benzylidene methylhexoside dibenzyl phosphate. It is well established that when the epoxide ring of a 2 : 3-anhydro-sugar is opened by acid or alkaline reagents the entering group may become attached to either of the carbon atoms forming part of the three-membered ring, a Walden inversion occurring at the point of attachment. Thus, Newth, Overend, and Wiggins (*J.*, 1947, 10) showed that treatment of (II) with hydrochloric or hydrobromic acid gave an α-methyl-2-deoxy-2-halogeno-D-altroside and an α-methyl-3-deoxy-3-halogeno-α-D-glucoside, the latter compound predominating in each case. It was, therefore, to be expected that the neutral resin obtained from (II) on reaction with dibenzyl hydrogen phosphate would be a mixture of 4 : 6-benzylidene α-methyl-D-altropyranoside-2 dibenzyl phosphate and 4 : 6-benzylidene α-methyl-D-glucopyranoside-3 dibenzyl phosphate. Benzyl and benzylidene groups were removed from the product by hydrogenolysis to give a mixture of methylglycoside phosphates. Chromatography of the mixture as ammonium salts on acid-washed paper showed the absence of inorganic phosphate and the presence of two organic phosphorus-containing compounds, one of which was decomposed by treatment with

periodate, the other being unaffected. This is in accordance with the expected behaviour of a mixture of methylaltroside-2 phosphate (III) and methylglucoside-3 phosphate (IV), since only the former contains a free 1 : 2-glycol system. Measurement of the amount of periodate consumed by the mixture indicated that it contained about four times as much of the altroside-2 phosphate as the glucoside-3 phosphate, a finding which is surprising in view of the above-mentioned observations of Newth, Overend, and Wiggins (*loc. cit.*). Acid hydroysis of the mixed phosphates yielded a mixture of two sugars identified as altrose and glucose. An attempt was made to separate the mixture of phosphates by using the chromatopile technique; although the separation was not complete, it was possible to isolate the major component, α-methyl-D-altropyranoside-2 phosphate (III) as its amorphous barium salt.

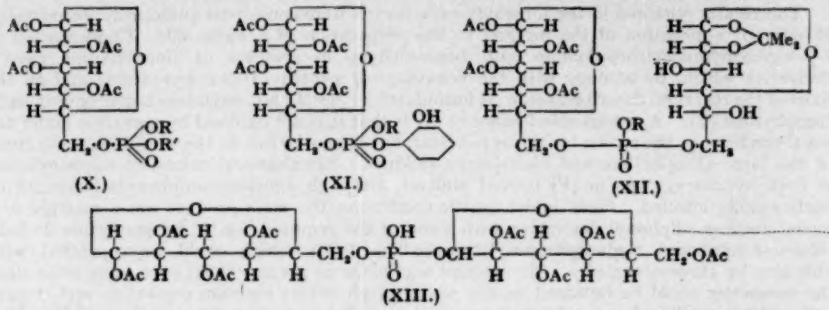
The results obtained in the foregoing experiments were considered sufficiently encouraging to warrant application of the method to the preparation of a nucleotide. Condensation of 7-β-D-glucopyranosyltheophylline with benzaldehyde in presence of zinc chloride gave a derivative which, by analogy with the behaviour of α-methyl-D-glucopyranoside and on the basis of the reactions described below, is formulated as 7-β-(4 : 6-benzylidene D-glucopyranosyl)-theophylline (V). A remarkable feature of (V) is that it is not oxidised by periodate under the usual conditions; the reason for this is not clear, but it may be due to the combined steric effect of the large theophylline and benzylidene residues. Simultaneous toluene-*p*-sulphonylation of both hydroxy-groups in (V) proved difficult, although dimethanesulphonylation could be fairly readily effected. Even under drastic conditions, the main product was a mixture of a monotoluene-*p*-sulphonyl derivative with some of the required 7-β-(4 : 6-benzylidene 2 : 3-di-toluene-*p*-sulphonyl D-glucopyranosyl)theophylline (VII) which could be separated with difficulty by chromatography. No attempt was made to prepare (VII) on a large scale since the monoester could be obtained readily under much milder reaction conditions and it gave with sodium methoxide an anhydro-compound identical with that prepared from (VII). The monotoluene-*p*-sulphonyl derivative must therefore be 7-β-(4 : 6-benzylidene 3-toluene-*p*-sulphonyl D-glucopyranosyl)theophylline (VI) and the anhydro-compound must be 7-β-(4 : 6-benzylidene 2 : 3-anhydro-D-allopyranosyl)theophylline (VIII), since there is no recorded instance of a 2 : 3-toluene-*p*-sulphonyl glucose derivative yielding a 2 : 3-anhydro-sugar with the mannose configuration and since, in forming an anhydro-sugar from a monotoluene-*p*-sulphonyl derivative, a Walden inversion occurs at the position originally occupied by the toluene-*p*-sulphonyl group.

The anhydro-glycoside (VIII) in dioxan solution was subjected to prolonged heating with dibenzyl hydrogen phosphate at 100°. The crystalline product which separated from the solution was soluble in water and strongly acidic. Analysis and potentiometric titration indicated that both benzyl and benzylidene groups had been lost, presumably by hydrolysis due to small amounts of water in the dioxan used. Paper chromatography showed that the product was homogeneous and it did not react with periodate under conditions known to be satisfactory for the oxidation of natural nucleotides. This product is therefore formulated as 7-β-D-glucopyranosyltheophylline-3′ phosphate (IX). No other phosphate was detected although in view of the small scale on which the reaction was carried out the possibility that some isomeric 2′-phosphate is also produced cannot be excluded.

As a further extension of these studies, and to provide suitable model experiments for eventual dinucleotide syntheses, it was decided to attempt the preparation of esters of phosphoric acid containing two different carbohydrate residues. No compounds of this nature had hitherto been prepared, but it seemed that some examples might be obtained from the reaction of 2 : 3-*iso*propylidene 5 : 6-anhydro-α-D-glucofuranose (I) with sugar phosphates. Accordingly, 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 dibenzyl phosphate (X; R = R′ = $PhCH_2$) was prepared from 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose and dibenzyl chlorophosphonate in the usual manner, and from it the crystalline 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 benzyl hydrogen phosphate (X; R = H, R′ = $PhCH_2$) was readily prepared by reaction with 4-methyl-morpholine (cf. Baddiley, Clark, Michalski, and Todd, *loc. cit.*) or lithium chloride (cf. Clark and Todd, *J.*, 1950, 2030) and characterised as its lithium and *cyclo*hexylamine salts. Complete removal of the benzyl groups from (X; R = R′ = $PhCH_2$) by hydrogenolysis gave 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 phosphate (X; R = R′ = H), identical with the material described by Lardy and Fischer (*J. Biol. Chem.*, 1946, **164**, 513).

Before condensation of the monobenzyl phosphate (X; R = H, R′ = $PhCH_2$) with (I) was attempted, its reaction with *cyclo*hexene oxide was examined. In chloroform solution reaction proceeded rather slowly but it furnished (1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6) benzyl 2-hydroxy*cyclo*hexyl phosphate (XI; R = $PhCH_2$) in fair yield as a crystalline solid. Removal

of the benzyl group by hydrogenation in dioxan solution gave the resinous 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 2′-hydroxy*cyclo*hexyl hydrogen phosphate (XI; R = H) characterised as its crystalline *cyclo*hexylamine salt. A solution of equimolecular quantities of (X; R = H, R′ = $PhCH_2$) and 1 : 2-*iso*propylidene 5 : 6-anhydro-α-D-glucofuranose (I) in chloroform was now heated under reflux for 45 minutes then set aside at room temperature for a day. The crystalline reaction product was stable to periodate and gave analytical values indicating that it was the expected (1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6) (1 : 2-*iso*propylidene α-D-glucofuranose-6) benzyl phosphate (XII; R = $PhCH_2$). Catalytic hydrogenation of this product gave (1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6) (1 : 2-*iso*-



(X.) (XI.) (XII.)

(XIII.)

propylidene α-D-glucofuranose-6) hydrogen phosphate (XII; R = H), characterised as its *cyclo*hexylamine salt, and the same product was also obtained by reaction of (X; R = R′ = H) with (I) in dioxan solution. As another example of a diester of phosphoric acid containing two sugar residues (1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6) (2 : 3 : 4 : 6-tetra-acetyl β-D-glucopyranose-1) hydrogen phosphate (XIII) was prepared as its crystalline *cyclo*hexylamine salt by treating the silver salt of (X; R = H, R′ = $PhCH_2$) with acetobromoglucose and hydrogenating the neutral reaction product.

The general conclusion drawn from the work described in this paper is that the epoxide route offers a feasible route to dicarbohydrate esters of phosphoric acid and to compounds of the nucleotide type. It is, however, severely limited in its application by the accessibility of appropriate anhydro-compounds, the tendency to formation of more than one product from other than 5 : 6-anhydro-sugar derivatives, and by the stereochemical considerations referred to earlier.

Experimental.

Reaction of 1 : 2-iso*Propylidene* 5 : 6-*Anhydro*-α-D-*glucofuranose* (I) *with Dibenzyl Hydrogen Phosphate.*—The anhydro-sugar (I) (1 g., 1 mol.) (Ohle and Vargha, *Ber.*, 1929, **62**, 2435) and dibenzyl hydrogen phosphate (1·38 g., 1 mol.) (prepared according to Clark and Todd, *J.*, 1950, 2023) were dissolved in carbon tetrachloride (75 c.c.), then heated to boiling, and *ca.* one-third of the solvent distilled off to remove traces of water. The residual liquid was now heated under reflux for 24 hours, moisture being excluded. The solution, now only faintly acidic, was washed with aqueous sodium hydrogen carbonate and then water, dried, and evaporated. The almost colourless gum so obtained was dissolved in chloroform and chromatographed on neutral alumina. A small amount of oily material was washed through with chloroform, and the main bulk of product was eluted with chloroform–methanol (50 : 1). Evaporation of the eluate gave 1 : 2-iso*propylidene* α-D-*glucofuranose*-6 *dibenzyl phosphate* as a colourless glass which could not be crystallised (Found: C, 55·0; H, 6·4. $C_{23}H_{29}O_9P,H_2O$ requires C, 55·4; H, 6·3%). Hydrolysis with aqueous-alcoholic sulphuric acid (N.) at room temperature yielded the known glucose-6 phosphate, isolated as its barium salt ($[\alpha]_D^{18} = +17·3°$; *c*, 2·55 in water).

In a second experiment the crude dibenzyl ester was hydrogenated in ethanol (100 c.c.) by means of a palladised charcoal catalyst (0·5 g.; 10%). When absorption of hydrogen ceased the filtered solution was evaporated under reduced pressure and the gummy residue was dissolved in water and neutralised with barium hydroxide, and the solution saturated with carbon dioxide, heated to boiling, and concentrated under reduced pressure to small volume (3—4 c.c.). The solution was filtered and poured into ethanol (200 c.c.), and the precipitated amorphous barium salt (0·7 g.) collected. It had $[\alpha]_D^{18} = +3·8°$ (*c*, 4·98 in water) and, as it only reduced Fehling's solution very slightly, it probably consisted mainly of barium 1 : 2-*iso*propylidene α-D-glucofuranose-6 phosphate. Without further purification the *iso*propylidene group was removed by hydrolysis, giving glucose-6 phosphate.

When the above dibenzyl ester (2·4 g.) was heated with 4-methylmorpholine (25 c.c.) at 100° for 2 hours an acidic amorphous material was obtained which gave analytical values suggesting that it was

the expected 1 : 2-iso*propylidene* α-D-*glucofuranose*-6 *benzyl hydrogen phosphate* (Found : C, 48·0; H, 6·4. $C_{16}H_{23}O_9P,\frac{1}{2}H_2O$ requires C, 48·1; H, 6·1%). This product gave a hygroscopic *cyclo*hexylamine salt which could not be crystallised.

Reaction of 4 : 6-*Benzylidene* α-*Methyl*-2 : 3-*anhydro*-D-*allopyranoside* (II) *with Dibenzyl Hydrogen Phosphate.*—A solution of the anhydro-glycoside (II) (5·3 g., 1 mol.) (Rosenfeld, Richtmeyer, and Hudson, *J. Amer. Chem. Soc.*, 1948, **70**, 2201) and dibenzyl hydrogen phosphate (5·6 g., 1 mol.) in carbon tetrachloride (250 c.c.) was distilled until about 50 c.c. of distillate had been collected, then heated under reflux for 7 days with exclusion of moisture. At intervals aliquots (2 c.c.) were removed and titrated with N/10-sodium hydroxide; it was observed that about 50% of the original acid was used up in 24 hours and about 95% in 7 days. The cooled solution was filtered, washed first with dilute sodium hydroxide then with water, dried, and evaporated. The residue (10 g.), a colourless glass, defied all attempts at crystallisation. A small sample (43 mg.) was dissolved in benzene–chloroform (20 c.c.; 1 : 1) and chromatographed on neutral alumina (3 g.). Elution with chloroform–methanol (100 : 1) yielded a colourless glass which had the correct analysis for a mixture of benzylidene methylhexoside dibenzyl phosphates (Found : C, 62·1; H, 5·8. Calc. for $C_{34}H_{33}O_9P$: C, 62·0; H, 5·8%).

The crude product obtained as above (6·3 g.) was dissolved in ethanol (200 c.c.) and hydrogenated at room temperature and atmospheric pressure by aid of a palladised charcoal catalyst (0·5 g.; 10%). Hydrogenation was slow and ceased after the hydrogen uptake corresponded to 3·5 mols. Catalyst was removed by filtration, the filtrate evaporated under reduced pressure, and the residual syrup dissolved in water (150 c.c.). Saturated aqueous barium hydroxide was added until the mixture was just alkaline to phenolphthalein, the mixture was saturated with carbon dioxide, then boiled, filtered, and concentrated to small bulk (50 c.c.) under reduced pressure. After removal of a small precipitate by centrifugation the solution was poured into acetone (300 c.c.). After several hours the precipitated mixture of barium α-methyl-D-altroside-2 phosphate (III) and barium α-methyl-D-glucoside-3 phosphate (IV) was collected. The mixture was purified by dissolution in water and reprecipitation with acetone giving a white amorphous powder (3·55 g.) (Found : C, 20·9; H, 4·0; P, 7·2. Calc. for $C_7H_{13}O_9PBa$: C, 20·5; H, 3·2; P, 7·6%).

A portion of the crude initial reaction product (1·3 g.) was hydrogenated as before and the resulting gum was heated for 6 hours under reflux with dilute sulphuric acid (25 c.c.; 2N.) then diluted to 100 c.c. with water, and barium carbonate added gradually until the solution was neutral. The mixture was heated to boiling and filtered from precipitated barium salts. The filtered solution was passed successively through short columns of Zeocarb 215 and Deacidite B to remove inorganic material and then concentrated to small bulk (10 c.c.). Paper chromatography of this solution by means of a collidine–water system with ammoniacal silver nitrate as spraying agent revealed the presence of two sugars which were identified as glucose and altrose by comparison with authentic samples run simultaneously. The altrose used was prepared from a sample of 4 : 6-benzylidene α-methyl-D-altroside kindly supplied by Prof. M. Stacey, to whom our thanks are due. The R_F value for altrose in collidine–water was 0·53 (corrected to 20° by using glucose, R_F 0·39, as standard). A small sample of the solution of altrose and glucose obtained as above was evaporated, and the residual gum taken up in a few drops of water and heated on the water-bath for 2 hours with phenylhydrazine acetate. The crystalline precipitate, recrystallised several times from aqueous ethanol, gave altrose phenylosazone, m. p. 178—179°.

Periodate Oxidation of Mixed Phosphates.—The above mixture of barium salts of methylaltroside-2 phosphate and methylglucoside-3 phosphate (97·5 mg.) was dissolved in water (3 c.c.), sodium sulphate (50 mg. in 1 c.c. water) added, and barium sulphate removed by centrifugation, the precipitate being washed thoroughly with hot water. The combined filtrate and washings were now treated with sodium metaperiodate solution (5 c.c.; 0·245M.), the volume was made up to 25 c.c., and the solution set aside at room temperature and the course of the oxidation followed in the usual way. The uptake of periodate was at first rapid up to 0·8 mol./mol. Subsequently a very slow further uptake occurred, the total absorption after 24 hours being 0·9 mol./mol. This result indicates that the original mixture contained approx. 80% of barium α-methyl-D-altroside-2 phosphate and 20% of barium α-methyl-D-glucoside-3 phosphate.

Barium α-*Methyl*-D-*altropyranoside*-2 *Phosphate* (III).—Dilute sulphuric acid (*ca.* 4 c.c.; N/2) was added to a solution of the mixed barium methylglycoside phosphates (400 mg.) prepared as described above so as to remove barium ions as sulphate. The barium-free solution was neutralised with dilute ammonia solution and a sample was chromatographed on acid-washed paper by Hanes and Isherwood's method (*Nature*, 1949, **164**, 1107), propanol–ammonia–water (6 : 3 : 1) being used as solvent system. Treatment of the chromatogram with the phosphate reagent described by these authors showed the presence of two main spots corresponding to the two sugar phosphates. Of these, that with the lower R_F value, 0·25, was evidently α-methyl-D-altroside-2 phosphate, since it was considerably more intense and it was absent if the mixture was treated with periodate before chromatography. The other spot, R_F 0·32, was stable to periodate and evidently was due to α-methyl-D-glucoside-3 phosphate. Although the separation of the two phosphates in this way was quite clear-cut, the low R_F values suggested that separation and isolation on any scale on a cellulose column would be very laborious. Recourse was therefore had to separation by using the chromatopile described by Mitchell and Haskins (*Science*, 1949, **110**, 278). In setting up the chromatopile the filter-papers were washed first with acetic acid (2N.) then with water until free from acid and dried at 60° before use. The mixed phosphates (from 350 mg. of the mixed barium salts) in the form of ammonium salts were applied on 13 papers, the chromatopile allowed to come to equilibrium in the presence of solvent (propanol–ammonia–water; 6 : 3 : 1) for 72 hours, and then run for 28 hours, by which time the solvent front had almost reached the bottom of the pile. The papers were now dried at 60°, and the position of the phosphates determined by spraying each fifteenth paper with phosphate reagent. All phosphate-containing material was found concentrated in the first 300 papers which were divided into groups of 15 sheets, each group being separately extracted with

water in a small Soxhlet and the extract concentrated to small bulk (2—3 c.c.). Small test paper chromatograms on each extract showed that a number of the fractions contained only the altrose derivative but none contained only the glucose derivative. The fractions (papers 81—170) containing only the altrose compound were combined and passed through a short column of Zeocarb 215 which was washed with water until the washings were no longer acidic. The combined washings (20 c.c.) were made alkaline (phenolphthalein) with barium hydroxide solution, saturated with carbon dioxide, boiled, and filtered. The filtrate was concentrated to small bulk (2 c.c.) under reduced pressure and poured into acetone (100 c.c.). *Barium α-methyl-*D*-altropyranoside-2 phosphate* separated as a white powder (90 mg.). It was purified by precipitation from aqueous solution with acetone and then had $[\alpha]_D^{12} = +34°$ (*c*, 1·03 in water), unchanged by further treatment (Found: C, 19·6; H, 4·1. $C_7H_{13}O_9PBa,H_2O$ requires C, 19·7; H, 3·5%). On periodate oxidation the substance consumed 1·06 mols. of oxidant per mol. in 24 hours, and paper chromatography of the corresponding ammonium salt showed that it was homogeneous and free from inorganic phosphate.

7-β-(4 : 6-*Benzylidene* D-*Glucopyranosyl*)*theophylline* (V).—A mixture of 7-β-D-glucopyranosyltheophylline (10 g., 1 mol.; Fischer and Helferich, *Ber.*, 1914, **47**, 217), anhydrous zinc chloride (20 g., 5 mols.), and freshly distilled benzaldehyde (50 c.c.) was shaken for 18 hours. The viscous mixture was now shaken vigorously with an equal volume of water, and the benzaldehyde phase separated, washed twice with water, and set aside for 2—3 hours. The crystalline product (10 g., m. p. *ca.* 245°) which separated was collected and recrystallised several times from aqueous ethanol or aqueous Cellosolve, giving 7-β-(4 : 6-*benzylidene* D-*glucopyranosyl*)*theophylline* as colourless hexagonal plates, m. p. 272—273° (Found: C, 55·5; H, 4·9; N, 12·8. $C_{20}H_{22}O_7N_4$ requires C, 55·8; H, 5·1; N, 13·0%). The substance was unaffected by aqueous periodate at room temperature for 24 hours either in aqueous suspension or dissolved in ethanol or dioxan.

*Toluene-*p*-sulphonylation of* 7-β-(4 : 6-*Benzylidene* D-*Glucopyranosyl*)*theophylline*.—A solution of the above benzylidene glucoside (0·5 g., 1 mol.) and toluene-*p*-sulphonyl chloride (0·66 g., 3 mols.) in dry pyridine (15 c.c.) was kept at 40° for 3 days then heated to 100° for 2 hours. The cooled brownish solution was poured into a well-stirred mixture of crushed ice and water (200 c.c.). After 2 hours' further stirring, the precipitate (0·6 g.) was collected, washed with water, and recrystallised from aqueous Cellosolve, giving 7-β-(4 : 6-*benzylidene* 3-*toluene-*p*-sulphonyl* D-*glucopyranosyl*)*theophylline* (VI) as colourless plates, m. p. 232—233° (decomp.) (Found: C, 55·5; H, 4·8; N, 9·5; S, 5·3. $C_{27}H_{28}O_9N_4S$ requires C, 55·4; H, 4·8; N, 9·6; S, 5·5%). A sample of this material was recovered unchanged after 6 hours' heating with sodium iodide in acetone in a sealed tube at 110°.

The Cellosolve mother-liquors from the crystallisation of the monotoluene-*p*-sulphonyl compound were combined and evaporated, and the residue (200 mg.) dissolved in benzene (75 c.c.) and put on a column of neutral alumina (6 g.). Elution with benzene–acetone (10 : 1) yielded 7-β-(4 : 6-*benzylidene* 2 : 3-*ditoluene-*p*-sulphonyl* D-*glucopyranosyl*)*theophylline* (VII) which crystallised from aqueous acetone as colourless woolly needles, m. p. 242° (decomp.) (Found: C, 55·5; H, 4·8; N, 7·9. $C_{34}H_{34}O_{11}N_4S_2$ requires C, 55·3; H, 4·6; N, 7·6%).

7-β-(4 : 6-*Benzylidene* 2 : 3-*Dimethanesulphonyl* D-*Glucopyranosyl*)*theophylline*.—A mixture of the above benzylidene glucoside (2 g., 1 mol.), methanesulphonyl chloride (1·6 g., 3 mols.), and dry pyridine (50 c.c.) was set aside at 40° for 18 hours then poured into a mixture of ice and water (500 c.c.), and stirring continued for 2 hours. The precipitated solid (1 g.) was collected, washed with water, and recrystallised from aqueous dioxan. The *dimethanesulphonyl* derivative was thus obtained as colourless needles, m. p. 233—234° (decomp.) (Found: C, 45·0; H, 4·2; N, 9·5. $C_{22}H_{26}O_{11}N_4S_2$ requires C, 45·0; H, 4·5; N, 9·6%).

7-β-(4 : 6-*Benzylidene* 2 : 3-*Anhydro-*D*-allopyranosyl*)*theophylline* (VIII).—(1) Methanolic sodium methoxide (from 0·2 g. of sodium in 5 c.c. of methanol) was added to a solution of the above 2 : 3-ditoluene-*p*-sulphonyl compound (VII) (0·2 g.) in chloroform (10 c.c.), and the mixture set aside for 4½ days, during which time a white precipitate formed. Water (15 c.c.) was added, the mixture was thoroughly shaken, and the chloroform layer separated, washed again with water until the washings were neutral (3 × 8 c.c.), then dried and evaporated under reduced pressure. The residue of 7-β-(4 : 6-*benzylidene* 2 : 3-*anhydro-*D*-allopyranosyl*)*theophylline* crystallised on addition of ether; recrystallised from aqueous ethanol, the anhydro-compound formed long colourless needles, m. p. 225—226° (Found: C, 58·2; H, 4·7; N, 13·5. $C_{20}H_{20}O_6N_4$ requires C, 58·3; H, 4·9; N, 13·6%).

(2) The above preparation was repeated, 7-β-(4 : 6-benzylidene 3-toluene-*p*-sulphonyl D-glucopyranosyl)theophylline (VI) (0·2 g.) being used in place of the ditoluene-*p*-sulphonyl compound. The product was again 7-β-(4 : 6-benzylidene 2 : 3-anhydro-D-allopyranosyl)theophylline, m. p. and mixed m. p. 225—226° (Found: C, 58·3; H, 4·7%).

7-β-D-*Glucopyranosyltheophylline-3′ Phosphate* (IX).—A mixture of the above anhydro-alloside (VIII) (0·2 g., 1 mol.), dibenzyl hydrogen phosphate (0·135 g., 1 mol.), and dioxan (7 c.c.) was heated in a sealed tube at 100° for 8 days. The crystalline product which had separated (80 mg.; m. p. 210—211°) was collected and recrystallised from water containing a small amount of acetone, giving 7-β-D-*glucopyranosyltheophylline-3′ phosphate* as colourless needles, m. p. 218° (decomp.), both benzyl and benzylidene groups having apparently been removed during the reaction (Found: C, 37·5; H, 5·3. $C_{13}H_{19}O_{10}N_4P$ requires C, 37·0; H, 4·5%). Potentiometric titration showed the presence of a secondary phosphoryl dissociation p*K* 6·0, and paper chromatography, propanol–ammonia–water or 5% aqueous disodium hydrogen phosphate–*iso*amyl alcohol being used, showed only one spot. Treatment of the paper chromatogram with periodate (Buchanan, Dekker, and Long, *J.*, 1950, 3162) showed the absence of a 1 : 2-glycol grouping. The dioxan mother-liquors from the original reaction smelt strongly of benzaldehyde but did not seem to contain any other glycoside phosphate; they were not, however, investigated in detail.

1 : 2 : 3 : 4-*Tetra-acetyl* β-D-*Glucopyranose*-6 *Dibenzyl Phosphate* (X; R = R′ = $PhCH_2$).—To an ice-cold solution of dibenzyl chlorophosphonate (from 20 g. of dibenzyl phosphite) in 2 : 6-lutidine (15 c.c.) a solution of 1 : 2 : 3 : 4-tetra-acetyl β-D-glucose (10 g.) (*Org. Synth.*, **22**, 57) in 2 : 6-lutidine (40 c.c.) was added dropwise with vigorous stirring during 45 minutes. The temperature was allowed to rise slowly to 15° during 1½ hours, and stirring continued at this temperature for a further 1½ hours. The stirred mixture was now cooled in ice, water (10 c.c.) was added dropwise, and after 45 minutes' stirring more water (100 c.c.) was added to dissolve the 2 : 6-lutidine hydrochloride and the solution was extracted with ice-cold chloroform (3 × 100 c.c.). The combined extracts were washed thoroughly with cold hydrochloric acid (3 × 100 c.c.; 3N.), then with sodium hydrogen carbonate, and finally with ice-water, dried, and evaporated. Ether (40 c.c.) was added to the residual oil, and the amorphous precipitate was filtered off and washed with a little ether. The combined filtrate and washings were evaporated, the residual syrup was taken up in methanol (35 c.c.), and water added until the mixture was opalescent. Set aside in the refrigerator overnight (more rapidly on being seeded), hydrated crystals of 1 : 2 : 3 : 4-*tetra-acetyl* β-D-*glucopyranose*-6 *dibenzyl phosphate* separated (6·3 g.); a further quantity (1·1 g.) was obtained by evaporating the mother-liquor, dissolving the residue in benzene (100 c.c.), shaking the solution with neutral alumina (10 g.), then evaporating, and crystallising the residue as before from aqueous methanol. Recrystallised from aqueous methanol, the ester formed colourless hydrated needles, m. p. 89—90° (Found: C, 54·0; H, 5·5; loss at 105°/0·1 mm., 2·6. $C_{33}H_{33}O_{13}P,H_2O$ requires C, 53·7; H, 5·6; H_2O, 2·9%). The ester had $[\alpha]_D^{25}$ +24·4° (*c*, 1·8 in methanol). The monohydrate slowly lost water *in vacuo* over phosphoric oxide at room temperature; the anhydrous ester could be recrystallised from dry ether and then had m. p. 105—107°.

A sample of the ester (1·22 g.) was hydrogenated in methanolic solution by aid of a palladium oxide catalyst. Catalyst was removed by filtration, the filtrate evaporated, and the residue (0·86 g.) recrystallised from methanol–light petroleum, giving 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 phosphate, m. p. 126—127° undepressed in admixture with a sample prepared by hydrogenating 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 diphenyl phosphate (Lardy and Fischer, *loc. cit.*).

(1 : 2 : 3 : 4-*Tetra-acetyl* β-D-*Glucopyranose*-6) *Benzyl Hydrogen Phosphate* (X; R = H, R′ = $PhCH_2$).—A solution of 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 dibenzyl phosphate (1 g.) and lithium chloride (0·35 g.) in ethoxyethanol (20 c.c.) was heated on a boiling-water bath for 2 hours. During the heating, crystalline material separated and a strong odour of benzyl chloride developed. The mixture was cooled, and the crystalline *lithium* salt (0·73 g., 88%) collected, washed with ethoxyethanol, and recrystallised from methylated spirit, from which it formed colourless needles, m. p. 270° (decomp.) (Found: C, 48·5; H, 5·0. $C_{21}H_{24}O_{13}PLi$ requires C, 48·1; H, 5·0%).

The above lithium salt (2·5 g.) was dissolved in water (50 c.c.), cooled to 0°, and acidified with ice-cold hydrochloric acid (6 c.c.). The oil which separated immediately crystallised on being shaken and was then collected and washed with a little ice-water. The yield of product was 1·68 g. and a further 0·5 g. was obtained from the mother-liquor by chloroform extraction. Recrystallised by slow addition of light petroleum (b. p. 60—80°) to its solution in cold ethoxyethanol, 1 : 2 : 3 : 4-*tetra-acetyl* β-D-*glucopyranose*-6 *benzyl hydrogen phosphate* forms colourless needles, m. p. 132—134° (Found: C, 48·4; H, 5·4. $C_{21}H_{27}O_{13}P$ requires C, 48·7; H, 5·3%). The monobenzyl ester is rather unstable and decomposes when its solution in ethoxyethanol is boiled or when it is heated for a time at 110°. It can also be prepared in a yield of 50—60% by heating the dibenzyl ester with 4-methylmorpholine for 2 hours at 100°; in this case it is most conveniently isolated as its cyclo*hexylamine* salt, which separates from water as fine colourless needles, m. p. 208—209° (Found: C, 52·4; H, 6·6; N, 2·3. $C_{27}H_{40}O_{13}NP$ requires C, 52·5; H, 6·5; N, 2·3%).

(1 : 2 : 3 : 4-*Tetra-acetyl* β-D-*Glucopyranose*-6) *Benzyl* 2-*Hydroxy*cyclo*hexyl Phosphate* (XI; R = $PhCH_2$).—The *cyclo*hexylamine salt of 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 benzyl hydrogen phosphate (0·5 g.) was dissolved in water (70 c.c.), and the solution cooled to 0°, acidified with ice-cold sulphuric acid (2 c.c.; N.), and rapidly extracted with ice-cold chloroform (3 × 25 c.c.). The extract was dried and evaporated and the free monobenzyl phosphate so obtained was dissolved in dry alcohol-free chloroform (10 c.c.). To this solution freshly distilled *cyclo*hexene oxide (0·2 g.) was added, and the mixture set aside for 5 days with exclusion of moisture. The solution was now washed with dilute aqueous sodium carbonate, then with water, and dried, and solvent and unchanged *cyclo*hexene oxide removed by evaporation under reduced pressure. The partially crystalline residue was taken up as far as possible in benzene, the solution filtered, and the filtrate evaporated, leaving a residue (0·47 g.) which was dissolved in hot methanol. Water was added until turbidity appeared; the solution was clarified again by addition of a few drops of methanol, boiled with charcoal, filtered hot, and set aside. On cooling the *ester* (XI; R = $PhCH_2$) separated as colourless needles (0·38 g.), m. p. 76—77° (Found: C, 52·4; H, 6·3. $C_{27}H_{37}O_{14}P$ requires C, 52·6; H, 6·1%).

(1 : 2 : 3 : 4-*Tetra-acetyl* β-D-*Glucopyranose*-6) 2-*Hydroxy*cyclo*hexyl Hydrogen Phosphate* (XI; R = H).—The neutral ester (XI; R = $PhCH_2$) (0·175 g.) was dissolved in purified dioxan (20 c.c.) and hydrogenated in presence of a palladium oxide catalyst at room temperature. Hydrogenation was complete in 2 hours, the catalyst was filtered off, and the filtrate evaporated under reduced pressure at room temperature. The resinous acidic residue was dissolved in acetone (5 c.c.), and a solution of *cyclo*hexylamine (50 mg.) in light petroleum (b. p. 40—60°) added. The gelatinous precipitate was collected and washed with acetone. It was now dissolved in water (5 c.c.), and the solution boiled with charcoal, filtered, and again evaporated to dryness under reduced pressure. The residual cyclo*hexylamine* salt was recrystallised from ethanol–acetone and formed colourless needles (0·12 g., 70%), m. p. 204—205° (decomp.); the salt was readily soluble in water, methanol, and ethanol and insoluble in ether, acetone, and light petroleum (Found: C, 50·2; H, 7·4; N, 2·6. $C_{33}H_{44}O_{14}NP$ requires C, 49·9; H, 7·1; N, 2·2%).

(1 : 2 : 3 : 4-*Tetra-acetyl* β-D-*Glucopyranose*-6)(1 : 2-iso*Propylidene* α-D-*Glucofuranose*-6) *Benzyl*

Phosphate (XII; R = $PhCH_2$).—The *cyclo*hexylamine salt of 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 benzyl hydrogen phosphate (X; R = H, R′ = $PhCH_2$) (0·75 g.) was converted into the free acid as before, and the latter dissolved in dry alcohol-free chloroform (5 c.c.). 1 : 2-*iso*Propylidene 5 : 6-anhydro-α-D-glucofuranose (I) (0·4 g.) was added, and the solution heated under reflux for 45 minutes then set aside at room temperature for 24 hours, moisture being excluded. The chloroform solution was now washed with aqueous sodium hydrogen carbonate then water, dried, and evaporated under reduced pressure at room temperature. The syrupy residue was dissolved in ethanol (15 c.c.), and the solution filtered from a small precipitate and concentrated to small bulk (3—4 c.c.). The crude *ester* was now recrystallised from methanol and yielded fine colourless needles (0·38 g.), m. p. 169—170° (Found: C, 49·8; H, 5·8; P, 4·0. $C_{26}H_{41}O_{18}P$ requires C, 50·0; H, 5·7; P, 4·3%). The preparation was repeated with the following variations: (*a*) use of half the amount of anhydro-sugar (yield, 0·21 g.); (*b*) use of double the amount of anhydro-sugar (yield, 0·4 g.); (*c*) quantities as before but the solution refluxed for 3 hours (decomposition occurred and no crystalline product was isolated).

The product was soluble in chloroform, methanol, dioxan, and benzene and insoluble in water and light petroleum. In aqueous dioxan solution, the compound was stable to sodium metaperiodate; after 24 hours at room temperature the uptake of oxidant was only 0·04 mol./mol.

(1 : 2 : 3 : 4-*Tetra-acetyl* β-D-*Glucopyranose*-6)(1 : 2-iso*Propylidene* α-D-*Glucofuranose*-6) *Hydrogen Phosphate* (XII; R = H).—(*a*) The above benzyl ester (135 mg.) was hydrogenated in dioxan solution (15 c.c.) by use of a palladium oxide catalyst (50 mg.) at room temperature and pressure. Hydrogenation was complete in 40 minutes, the hydrogen uptake (4·5 c.c.) being slightly more than the theoretical for 1 mol. Catalyst was removed by filtration, the filtrate evaporated under reduced pressure, and the crystalline residue of the *acid ester* (XII; R = H) (0·114 g.) washed thoroughly first with dioxan–light petroleum then with light petroleum alone, and dried at room temperature over phosphoric oxide and paraffin wax (Found: C, 43·3; H, 5·9. $C_{19}H_{31}O_{18}P$ requires C, 43·8; H, 5·6%). The product had a rather indefinite m. p., 132—135°, which was not affected by reprecipitation from dioxan solution with light petroleum; it was rather unstable, decomposed slowly when kept, and could not be recrystallised from boiling solvents.

When the product (100 mg.) was dissolved in dioxan (10 c.c.) and a solution of *cyclo*hexylamine (50 mg.) in dioxan (5 c.c.) added, a gelatinous precipitate was formed. This was collected and recrystallised from ethanol, giving the cyclo*hexylamine* salt (102 mg.) as colourless needles, m. p. 217° (decomp.) (Found: C, 47·7; H, 6·9; N, 2·0. $C_{25}H_{48}O_{18}NP$ requires C, 47·7; H, 6·6; N, 1·9%).

(*b*) A solution of 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 phosphate (X; R = R′ = H) (535 mg.) and 1 : 2-*iso*propylidene 5 : 6-anhydro-α-D-glucofuranose (I) (265 mg.) in anhydrous dioxan was set aside for 7 days at room temperature. The slightly turbid solution was filtered and evaporated under reduced pressure. The oily residue was dissolved in ice-cold chloroform (50 c.c.), the solution extracted with ice-cold aqueous sodium hydrogen carbonate (50 c.c. of 1%), and the extract acidified with ice-cold sulphuric acid (10 c.c.; N.) and rapidly extracted with ice-cold chloroform (240 c.c.). The chloroform extract was washed with water, dried (Na_2SO_4) for 30 minutes, and evaporated. The residue was dissolved in dioxan (10 c.c.), and *cyclo*hexylamine (100 mg.) in dioxan (5 c.c.) added. The precipitated crude *cyclo*hexylamine salt (453 mg.) was thrice recrystallised from ethanol and then formed colourless needles (134 mg.), m. p. 216—217°, undepressed in admixture with the *cyclo*hexylamine salt prepared as described under (*a*).

(1 : 2 : 3 : 4-*Tetra-acetyl* β-D-*Glucopyranose*-6)(2 : 3 : 4 : 6-*Tetra-acetyl* β-D-*Glucopyranose*-1) *Hydrogen Phosphate* (XIII).—A mixture of α-acetobromoglucose (0·4 g.), silver 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 benzyl phosphate (X; R = H, R′ = $PhCH_2$) (1·2 g.), and dry benzene (30 c.c.) was warmed to 50° for 30 minutes in a flask fitted with a reflux condenser and an efficient mechanical stirrer. The mixture was now refluxed for 40 minutes with continued vigorous stirring. The dark-coloured solution was filtered from silver bromide, boiled for a few minutes with charcoal, then evaporated to dryness under reduced pressure. The syrupy residue could not be crystallised and so was hydrogenated directly in dioxan solution with a palladium oxide catalyst (100 mg.) to remove the benzyl group; hydrogen uptake (28 c.c.) was complete in 1½ hours. The filtered solution was evaporated at room temperature under reduced pressure, the residue taken up in chloroform (25 c.c.), and the solution extracted with ice-cold sodium hydrogen carbonate (30 c.c.; 1%). The aqueous layer was separated, acidified with ice-cold sulphuric acid (5 c.c.; N.), and quickly extracted with cold chloroform (2 × 30 c.c.). The chloroform extract was washed with ice-water, dried (Na_2SO_4) for 30 minutes, and evaporated under reduced pressure at room temperature. The yellowish syrupy residue was dissolved in dioxan (5 c.c.), and a solution of *cyclo*hexylamine (50 mg.) in light petroleum (10 c.c.; b. p. 60—80°) added. The precipitated salt was purified by dissolution in acetone–methanol (5 c.c.; 1 : 1) and addition of light petroleum (b. p. 40—60°) to turbidity; filtration and addition of more light petroleum then reprecipitated it. After three such treatments, the cyclo*hexylamine* salt of (1 : 2 : 3 : 4-*tetra-acetyl* β-D-*glucopyranose*-6)(2 : 3 : 4 : 6-*tetra-acetyl* β-D-*glucopyranose*-1) *hydrogen phosphate* was obtained as a white microcrystalline powder (120 mg.), m. p. 204—206° (decomp.) (Found: C, 47·3; H, 6·5; N, 1·8. $C_{34}H_{53}O_{26}NP$ requires C, 47·6; H, 6·1; N, 1·6%).

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502. *Carbazoles, Carbolines, and Related Compounds. Part IV.* Amino-derivatives of 9-Arsafluoreninic Acid.†*

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The chemistry of 9-arsafluoreninic acid † (V) has been extended by the preparation of the 2-, 3-, and 4-amino-derivatives. The 2 : 7-dinitro-derivative (IX; R = NO_2) has been prepared, but its reduction to (IX; R = NH_2) has not been accomplished.

Morgan and Stewart (*J.*, 1931, 620; 1932, 1634; 1933, 1454) have shown that the introduction of an arsonic acid radical and an amino-group into the fluorene molecule leads to the appearance of trypanocidal properties. Extending these observations, we have now prepared some amino-derivatives of 9-arsafluoreninic acid † (V), in which the arsenic acid radical has been transferred to an intracyclic position.

9-Arsafluoreninic acid (V), the parent compound of the group, was prepared by Aeschlimann, Lees, McCleland, and Nicklin (*J.*, 1925, **127**, 66) by subjecting 2-aminodiphenyl to the Bart reaction, whereby diphenylyl-2-arsonic acid (I) was produced, followed by ring closure with sulphuric acid. Experimental details for the preparation of (I) were not given. We have, therefore, carried out a systematic study of the methods whereby the latter compound may be obtained, and find Scheller's modification of the Bart reaction, as described by Oneto and Way (*J. Amer. Chem. Soc.*, 1941, **63**, 3068), to be entirely satisfactory. Our results are thus at variance with Cookson and Mann's observations (*J.*, 1949, 2897).

Nitration of the acid (V) with one equivalent of nitric acid in concentrated sulphuric acid led to formation of the 2-nitro-derivative (VIII; R = NO_2), reduced by ammonical ferrous sulphate (Jacobs, Heidelberger, and Rolf, *J. Amer. Chem. Soc.*, 1918, **40**, 1585) to the 2-amino-compound (VIII; R = NH_2).

Preliminary attempts to confirm the constitution assigned to (VIII; R = NH_2), and hence to (VIII; R = NO_2), by its alternative synthesis from 2-amino-4′-nitrodiphenyl (Scarborough and Waters, *J.*, 1927, 89), proved unsuccessful. The latter compound readily gave 4′-nitrodiphenylyl-2-arsonic acid (VII; R = NO_2) in excellent yield when submitted to the Scheller–Bart reaction, but attempts to effect ring closure of this compound with concentrated sulphuric acid invariably led to recovery of unchanged material. Heating with phosphorus oxychloride likewise failed to enforce cyclisation, though in this instance a product was obtained which was undoubtedly a di-(4′-nitro-2-diphenylyl)arsinic acid (IV), formed by loss of arsenic acid between two molecules of 4′-nitrodiphenylyl-2-arsonic acid. A similar transformation has been recorded by Morgan and Davies (*Proc. Roy. Soc.*, 1930, **127**, *A*, 1) in the diphenylyl-2-stibonic acid series.

Attention was, therefore, directed to the ring closure of 4′-aminodiphenylyl-2-arsonic acid derivatives, whereby it was hoped that the inhibitory influence of the 4′-nitro-group on the cyclisation of (VII; R = NO_2) (cf. Morgan and Walls, *J.*, 1932, 2225) would be avoided (cf. Petrow, *J.*, 1945, 18; Walls, *J.*, 1947, 67). The amino-compound required for this purpose was obtained by reduction of (VII; R = NO_2) with alkaline ferrous sulphate, or, more conveniently, by careful hydrogenation, Raney nickel being used (cf. Doak and Steinmann, *J. Amer. Chem. Soc.*, 1946, **68**, 1989). Experiments on its ring closure with concentrated sulphuric acid proved successful, authentic 2-amino-9-arsafluoreninic acid (VIII; R = NH_2) being produced, identical in every way with the product obtained from the 2-nitro-acid (above), the constitution of which is accordingly confirmed. The 2-amino-acid was also prepared by cyclisation of 4′-carbethoxyaminodiphenylyl-2-arsonic acid (VII; R = NH·CO_2Et), obtained by a Scheller–Bart reaction on 2-amino-4′-carbethoxyaminodiphenyl (Walls, *J.*, 1947, 67).

3-Nitro-9-arsafluoreninic acid (XI; R = NO_2) was obtained by ring closure of 5-nitrodiphenylyl-2-arsonic acid (X; R = NO_2) with concentrated sulphuric acid at 65°. The preparation of the latter compound was achieved through a Scheller–Bart reaction on 2-amino-5-nitrodiphenyl (Bell, *J.*, 1928, 2770). Reduction with alkaline ferrous sulphate furnished

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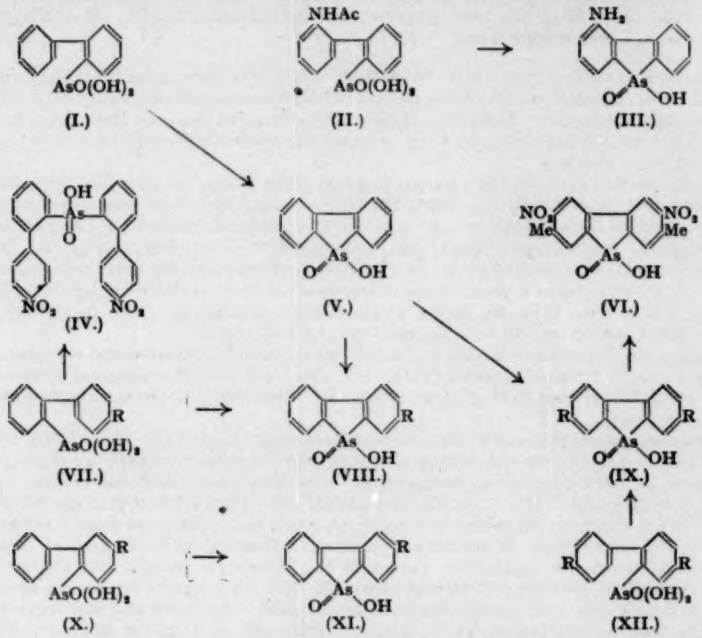
† The nomenclature used in this paper involves transference of the termination denoting the nature of the arsenic acid to the end of the name of the fundamental skeleton, with elimination of a terminal "e". For the use of the termination "inic" to denote acids of the type $R_2AsO{\cdot}OH$, compare the analogous phosphorus acids (*J.*, 1951, 1868, footnote). Ed.

3-amino-9-arsafluoreninic acid, also obtained by conversion of (X; R = NO_2) into 5-amino-diphenylyl-2-arsonic acid (X; R = NH_2), followed by careful ring closure with sulphuric acid.

The preparation of 4-amino-9-arsafluoreninic acid (III; R = NH_2) offered considerable experimental difficulty. 2-Amino-2′-nitrodiphenyl, required for this purpose, was first described by Mascarelli and Gatti (*Atti Accad. Lincei*, 1929, **10**, 441), who obtained it in impure condition by partial reduction of 2 : 2′-dinitrodiphenyl with aqueous-alcoholic ammonium sulphide. Its preparation was subsequently improved by Purdie (*J. Amer. Chem. Soc.*, 1941, **63**, 2276), but



this method likewise failed to give satisfactory results in our hands. Acetylation of the sticky product so obtained, however, gave a low yield of 2-acetamido-2′-nitrodiphenyl, which was directly reduced to 2-acetamido-2′-aminodiphenyl. The latter compound was also obtained, albeit in unsatisfactory yield, by partial acetylation of 2 : 2′-diaminodiphenyl as described by Sako (see *Chem. Abs.*, 1932, **26**, 3246). Conversion into 2′-acetamidodiphenylyl-2-arsonic acid proceeded fairly well, but cyclisation to 4-amino-9-arsafluoreninic acid (III) was accompanied by a very marked drop in yield.

Dinitration of (V) gave 2 : 7-dinitro-9-arsafluoreninic acid (IX; R = NO_2). The constitution assigned to this compound follows from the formation of (VIII; R = NO_2) by mononitration of (V), and from the established formation of 2 : 7-dinitrofluorenone from fluorenone (Schultz, *Annalen*, 1880, **203**, 104), an analogous ring system in which the 9-position is likewise occupied by an electrophilic group. The direct preparation of (IX; R = NO_2) from 4 : 4′-dinitrodiphenylyl-2-arsonic acid was not possible, however, as 2-amino-4 : 4′-dinitrodiphenyl (Ritchie, *Proc. Roy. Soc. N.S.W.*, 1945, **78**, 177) behaved abnormally in the Scheller–Bart reaction giving a mixture of 4 : 4′-dinitro- and 2-hydroxy-4 : 4′-dinitro-diphenyl.

Attempts to reduce 2 : 7-dinitro-9-arsafluoreninic acid by chemical methods proved uniformly unsuccessful owing to the virtual insolubility of the compound in the usual solvents. Failure likewise followed experiments employing catalytic techniques: the compound was recovered unchanged after its hot 1% solution in 2-ethoxyethanol had been shaken with palladised charcoal and hydrogen, and use of Raney nickel and hydrogen under the same experimental conditions led to formation of benzidine. A dinitrodiphenylyl-2-arsonic acid of unknown orientation,

obtained by nitration of diphenylyl-2-arsonic acid (I), likewise proved too insoluble for reduction to the corresponding diamine.

Attention was, therefore, directed to the possibility of synthesising 2 : 7-diamino-9-arsafluoreninic acid (IX; R = NH_2) by ring closure of 4 : 4′-diaminodiphenylyl-2-arsonic acid derivatives (XII; R = NH_2). Attempts to prepare compounds of the latter type, however, proved disappointing. Thus 2-amino-4 : 4′-biscarbethoxyaminodiphenyl (Walls, *J.*, 1947, 67) behaved abnormally both in the usual Bart reaction, in which *NN*′-dicarbethoxybenzidine was formed, and in the Scheller modification, *NN*′-dicarbethoxy-2-hydroxybenzidine being obtained. 4 : 4′-Dicarbethoxy- and 4 : 4′-dinitro-groups thus seem to exert a similar inhibitory influence upon the replacement of a 2-amino- by a 2-arsonic group in the diphenyl series. The reason for this anomalous behaviour is not evident, however, as previous observations, *inter al.*, by Walls (*loc. cit.*), would appear to indicate that the polar effects of a 4 : 4′-dinitro- may be reversed by conversion into a 4 : 4′-diamino-group.

Experimental.

M. p.s are uncorrected.

Diphenylyl-2-arsonic acid (I).—2-Aminodiphenyl (15 g.) in absolute ethanol (400 ml.) was treated with concentrated sulphuric acid (7 ml.), cooled to 5°, and the solution diazotised with a saturated solution of sodium nitrite (6·2 g.). The mixture, kept at 0—5°, was mechanically stirred for 2 hours, and arsenic trichloride (28 g.) in absolute ethanol (100 ml.) was then slowly added. After a further ½ hour cuprous bromide (2·0 g.) was added, and the mixture warmed gradually, with vigorous stirring, to 70°, at which temperature it was kept until evolution of nitrogen had ceased. Steam-distillation of the mixture removed ethanol and traces of diphenyl also formed in the reaction. Diphenylyl-2-arsonic acid crystallised on cooling, and was purified *via* the potassium salt and by crystallisation from 50% aqueous acetic acid, white needles (40%) being obtained having m. p. 206°. Aeschlimann *et al.* (*loc. cit.*) give m. p. 205° (uncorr.).

9-Arsafluoreninic Acid (V).—The foregoing compound (12 g.) was heated in concentrated sulphuric acid (90 ml.) for 1 hour at 50—60°, and the mixture was then poured slowly into ice-water. After some time, the precipitated material was collected and purified from glacial acetic acid. 9-Arsafluoreninic acid formed needles (80%), m. p. >300° (Found : C, 54·9; H, 3·5. Calc. for $C_{13}H_9O_2As$: C, 55·4; H, 3·5%).

2-Nitro-9-arsafluoreninic acid (VIII; R = NO_2).—9-Arsafluoreninic acid (2·15 g.), dissolved in concentrated sulphuric acid (6 ml.), was treated at 0° with a mixture of nitric acid (0·5 ml., 1·0 mol; *d* 1·42) and concentrated sulphuric acid (0·5 ml.), added dropwise with stirring. Stirring was continued for a further ½ hour, and the mixture was then poured on ice. The precipitate was collected, and crystallised from 50% aqueous acetic acid, giving *2-nitro-9-arsafluoreninic acid*, pale yellow crystals (90%), m. p. >345° (Found : C, 46·7; H, 2·7; N, 5·4. $C_{13}H_8O_4NAs$ requires C, 47·2; H, 2·6; N, 4·6%).

2-Amino-9-arsafluoreninic Acid (VIII; R = NH_2).—A solution of 2-nitro-9-arsafluoreninic acid (1·25 g.) in 2·5N-sodium hydroxide (25 ml.) was added in one portion to a solution of ferrous sulphate (8 g.) in water (25 ml.) which had been made just alkaline to litmus with aqueous ammonia. The mixture was shaken vigorously for 10 minutes and filtered, and the filtrate acidified to pH 3. The pink precipitate was collected and purified by reprecipitation to give *2-amino-9-arsafluoreninic acid* (75%), m. p. 240° (Found : C, 52·2; H, 3·9; N, 4·5. $C_{13}H_{10}O_2NAs$ requires C, 52·4; H, 3·6; N, 5·1%).

4′-Nitrodiphenylyl-2-arsonic acid (VII; R = NO_2), yellow crystals (77%) from methanol or aqueous acetic acid, had m. p. 290° (decomp.) (Found : C, 44·7; H, 3·3; N, 4·1. $C_{12}H_{10}O_5NAs$ requires C, 44·6; H, 3·1; N, 4·3%).

Di-(4′-nitro-2-diphenylyl)arsinic Acid (IV).—4′-Nitrodiphenylyl-2-arsonic acid (5 g.) was heated under reflux with phosphorus oxychloride (10 g.) for 1½ hours. Excess of halides was removed under reduced pressure, and the residue was extracted with 2N-sodium hydroxide solution. The resulting sodium salt was decomposed with dilute hydrochloric acid, and the solids (4·7 g.) thus formed were extracted with boiling ethanol (200 ml.). The insoluble fraction (1·1 g.) yielded *di-(4′-nitrodiphenylyl)-arsinic acid*, m. p. >260°, on crystallisation from ethanol (Found : C, 57·5; H, 4·1; N, 5·6. $C_{24}H_{17}O_6N_2As$ requires C, 57·2; H, 3·4; N, 5·6%).

4′-Aminodiphenylyl-2-arsonic acid (VII; R = NH_2).—(i) 4′-Nitrodiphenylyl-2-arsonic acid was reduced with ammoniacal ferrous sulphate, to give *4′-aminodiphenylyl-2-arsonic acid*, m. p. 212—213° (Found : N, 4·9. $C_{12}H_{12}O_3NAs$ requires N, 4·8%). (ii) The corresponding nitro-compound (10 g.) was dissolved in ethanol (150 ml.), and the mixture shaken with Raney nickel and hydrogen until the theoretical volume of gas had been absorbed. After filtration, the solution was concentrated under reduced pressure to a small bulk, water was added, and the mixture cooled. 4′-Aminodiphenylyl-2-arsonic acid separated in nearly quantitative yield as microcrystals, m. p. 213° (Found : N, 4·9%), not depressed on admixture with a sample prepared by method (i) above.

Ring closure of (VII; R = NH_2) gave (VIII; R = NH_2), m. p. 240°, not depressed on admixture with a sample prepared *via* nitroarsafluoreninic acid (see above).

4′-Carbethoxyaminodiphenylyl-2-arsonic acid (VII; R = NH·CO_2Et) formed crystals (15%), m. p. 228°, from aqueous ethanol (Found : C, 49·3; H, 4·6; N, 3·8. $C_{15}H_{16}O_5NAs$ requires C, 49·3; H, 4·4; N, 3·8%). Ring closure was effected by heating the arsonic acid (2 g.) with concentrated sulphuric

acid (15 ml.) at 125—130° for 20 minutes. After 30 minutes, the mixture was poured on ice. The precipitated product was collected and crystallised from aqueous ethanol, giving (VIII; R = NH_2) (50%), identical in m. p. and mixed m. p. with an authentic specimen.

5-Nitrodiphenylyl-2-arsonic acid (X; R = NO_2), prepared by a Scheller–Bart reaction on 2-amino-5-nitrodiphenyl, formed glistening plates (50%) (from aqueous ethanol), m. p. 228—229° (Found: C, 44·2; H, 3·1; N, 4·3. $C_{12}H_{10}O_5NAs$ requires C, 44·5; H, 3·1; N, 4·3%).

3-Nitro-9-arsafluoreninic acid (XI; R = NO_2), obtained by heating the foregoing compound (16 g.) with concentrated sulphuric acid (125 ml.) at 60° for 45 minutes, formed a crystalline powder (75%) from aqueous acetic acid and had m. p. >330° (Found: C, 47·4; H, 2·8; N, 4·7. $C_{12}H_8O_4NAs$ requires C, 47·2; H, 2·6; N, 4·6%).

3-Amino-9-arsafluoreninic Acid (XI; R = NH_2).—The foregoing compound (6·2 g.), suspended in sodium hydroxide solution (40 ml., 1 equiv.), was added to reduced iron (3·5 g.) in sodium chloride solution (7 ml. containing 2·6 g. of sodium chloride) at 90°. The mixture was kept at this temperature for 2 hours, sufficient hydrochloric acid being added from time to time to keep the pH at *ca.* 7—8. Sodium hydroxide solution (5 ml.; 33%) was then added, and the mixture heated to boiling and filtered hot. The residues were twice extracted with alkali, and the bulked filtrates acidified to pH 7. The precipitated material was collected, purified by reprecipitation, and finally crystallised from aqueous acetic acid, giving *3-amino-9-arsafluoreninic acid* (87%), orange needles, m. p. >350° (Found: C, 52·0; H, 3·3; N, 5·0. $C_{12}H_{10}O_2NAs$ requires C, 52·4; H, 3·6; N, 5·1%).

5-Aminodiphenylyl-2-arsonic Acid (X; R = NH_2).—(i) A solution of 5-nitrodiphenylyl-2-arsonic acid (13·3 g.) in sodium hydroxide (2·5—3N.) was added in one portion to ferrous sulphate (80 g., 7 equivs.) dissolved in water (250 ml.) and made alkaline with sodium hydroxide. The mixture was shaken vigorously for 15 minutes, precipitated iron salts were removed by centrifugation, and the liquors concentrated to 200 ml. Acidification to pH 3 precipitated *5-aminodiphenylyl-2-arsonic acid*, needles (66%) (from aqueous ethanol), m. p. 187° (Found: C, 49·3; H, 4·1; N, 5·3. $C_{12}H_{12}O_3NAs$ requires C, 49·2; H, 4·1; N, 4·8%). (ii) The foregoing amine was obtained (92% yield) by catalytic hydrogenation of the nitro-compound in ethanol, a Raney-nickel catalyst being used.

Cyclisation with concentrated sulphuric acid for 45 minutes at 60° gave (XI; R = NH_2) (80%), identical in m. p. and mixed m. p. with a specimen prepared by reduction of (XI; R = NO_2) (see above).

2′-Acetamidodiphenylyl-2-arsonic acid (II) was prepared from 2′-acetamido-2-aminodiphenyl by the Scheller–Bart reaction, and formed crystals (from ethanol), m. p. 122° (Found: C, 49·6; H, 4·3. $C_{14}H_{14}O_4NAs$ requires C, 50·2; H, 4·2%).

4-Amino-9-arsafluoreninic acid (III), obtained in very low yield by ring closure of the foregoing compound, formed microcrystals, m. p. >300° (Found: N, 5·3. $C_{12}H_{10}O_2NAs$ requires N, 5·4%).

2 : 7-Dinitro-9-arsafluoreninic Acid (IX; R = NO_2).—Nitration of 9-arsafluoreninic acid (18 g.) in concentrated sulphuric acid (50·0 ml.) with a mixture of nitric acid (8·4 ml.; *d* 1·42) and concentrated sulphuric acid (8·4 ml.) at 2°, gave *2 : 7-dinitro-9-arsafluoreninic acid* (87%) as a pale yellow powder, which was purified repeatedly through the potassium salt and then had m. p. >300° (Found: C, 40·6; H, 2·3; N, 7·9. $C_{12}H_7O_8N_2As$ requires C, 41·2; H, 2·0; N, 8·0%).

Dinitrodiphenylyl-2-arsonic Acid.—Diphenylyl-2-arsonic acid (13·4 g.) in concentrated sulphuric acid (45 ml.) was treated with a mixture of nitric acid (6·8 ml.; *d* 1·42) and concentrated sulphuric acid (6·8 ml.) added dropwise with stirring at 5°. After 1 hour the mixture was allowed to warm to room temperature and then added to a large volume of ice-water. The precipitated material was collected. Purification many times through the potassium salt yielded a *dinitrodiphenylyl-2-arsonic acid* (70%), m. p. >300° (Found: C, 39·7; H, 2·0; N, 7·4. $C_{12}H_8O_7N_2As$ requires C, 39·2; H, 2·5; N, 7·6%).

Scheller–Bart Reaction on 2-Amino-4 : 4′-dinitrodiphenyl.—2-Amino-4 : 4′-dinitrodiphenyl (4·45 g.) was submitted to the Scheller–Bart reaction, and the product triturated with warm acetone. The fraction insoluble in acetone, on purification from acetic acid, yielded *2-hydroxy-4 : 4′-dinitrodiphenyl*, yellow needles, m. p. 209—210° (Found: C, 55·5; H, 3·3; N, 10·6. $C_{12}H_8O_5N_2$ requires C, 55·4; H, 3·1; N, 10·7%), soluble in alkali. The soluble fraction, when kept in acetone, deposited yellow needles (3·2 g.) which, after repeated crystallisation from aqueous acetone, yielded 4 : 4′-dinitrodiphenyl, m. p. 235° alone or on admixture with an authentic specimen.

Bart Reaction on 2-Amino-4 : 4′-biscarbethoxyaminodiphenyl.—2-Amino-4 : 4′-biscarbethoxyaminodiphenyl (34·3 g.), suspended in 20% hydrochloric acid (70 ml.) and some water, was diazotised with sodium nitrite (7 g.) in water (20 ml.). The cold solution was added to 10% sodium hydroxide solution (250 ml.) at 0°, followed by sodium arsenite (17·5 g.) dissolved in water, and added in one portion with vigorous stirring. The temperature was then gradually raised to 65°, and kept thereat for 3 hours. The cooled solution was acidified with acetic acid, and the precipitated solids (14 g.; m. p. 200—204°) were collected and crystallised from aqueous ethanol, yielding *4 : 4′-dicarbethoxybenzidine*, m. p. 234—235° (Found: C, 65·8; H, 5·8; N, 8·7. $C_{18}H_{20}O_4N_2$ requires C, 65·8; H, 6·1; N, 8·5), not depressed on admixture with an authentic specimen. The latter was prepared by treating a just boiling solution of benzidine (47·6 g.) in ethanol (600 ml.) with diethylaniline (79 g.), followed dropwise by ethyl chloroformate (70 g.), added at such a rate that moderate refluxing ensued. Heating was discontinued after 2 hours, and the mixture, after being kept overnight, was poured into N-hydrochloric acid (1600 ml.). The precipitated solids were collected and crystallised from ethanol, giving *NN′*-dicarbethoxybenzidine as long needles (45 g.), m. p. 235—237° (Found: C, 65·5; H, 6·6; N, 8·6%).

Scheller–Bart Reaction on 2-Amino-4 : 4′-biscarbethoxyaminodiphenyl.—2-Amino-4 : 4′-biscarbethoxyaminodiphenyl (34·3 g.) was submitted to the Scheller–Bart reaction. The product (11 g.), isolated in the usual way, on purification from aqueous ethanol, yielded *4 : 4′-biscarbethoxyamino-2-hydroxydiphenyl*, needles, m. p. 128—130° (Found: C, 62·3; H, 5·9; N, 8·2. $C_{18}H_{20}O_5N_2$ requires C, 62·6; H, 5·5; N, 8·3%). The product was soluble in alkali.

Miscellaneous Experiments.—(i) By submitting 2-amino-4 : 4′-ditolyl (Marler and Turner, *J.*, 1932, 2391) to the Scheller–Bart reaction, 4 : 4′-*dimethyldiphenylyl-2-arsonic acid* (XII; R = Me) was obtained; it formed needles, m. p. 237°, from 75% acetic acid (Found: C, 54·7; H, 4·9. $C_{14}H_{15}O_3As$ requires C, 54·9; H, 4·9%). Extraction of the alkaline solution of the crude product with ether yielded 4 : 4′-ditolyl, pale yellow prisms (from light petroleum), m. p. 122—123° (Found: C, 92·0; H, 7·8. Calc. for $C_{14}H_{14}$: C, 92·3; H, 7·7%) not depressed in admixture with an authentic specimen (m. p. 125°).

(ii) Ring closure of 4 : 4′-dimethyldiphenylyl-2-arsonic acid with concentrated sulphuric acid furnished 2 : 7-*dimethyl*-9-*arsafluoreninic acid* (IX; R = Me), white needles (92%) (from acetic acid), m. p. 321—322° (Found: C, 57·8; H, 4·6. $C_{14}H_{11}O_3As$ requires C, 58·3; H, 4·5%).

(iii) Nitration of the foregoing compound (400 mg.) in concentrated sulphuric acid (4 ml.) with a mixture of nitric acid (0·13 ml.; *d* 1·42) and concentrated sulphuric acid (0·15 ml.) at 0—5° gave 2 : 7-*dimethyl*-3 : 6-*dinitro*-9-*arsafluoreninic acid* (VI), as feathery needles (76%) (from acetic acid), m. p. >300° (Found: N, 7·4. $C_{14}H_{11}O_8N_2As$ requires N, 7·4%). Attempts to reduce this compound to the corresponding diamino-derivative proved unsuccessful owing to its sparing solubility in suitable solvents.

(iv) 4′ : 5-*Dinitrodiphenylyl-2-arsonic acid*, m. p. 220° (decomp.) (Found: C, 39·6; H, 2·8; N, 7·8. $C_{12}H_9O_7N_2As$ requires C, 39·2; H, 2·5; N, 7·6%), was obtained in low yield from 2-amino-4′ : 5-dinitrodiphenyl. Attempts at ring closure were unsuccessful.

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503. *Structural Influences determining Tautomeric Equilibria in Phenylpropenes.*

By L. Bateman and J. I. Cunneen.

3-Alkyl substitution in 1-phenylpropene (II; R′ = R″ = H) leads to the non-styryl-conjugated isomer (I) forming a measurable proportion of the equilibrated mixture in alkaline methyl-alcoholic solution at 165°. The influence of different alkyl groups may be correlated with (*a*) the extent of CH-bond first-order hyperconjugation, and (*b*) the operation of a specific effect on replacement of the CH_3 group by CHR. The experimental data permit the magnitude of these factors and also the resonance energy of the styryl unit to be evaluated. These quantities, and reasonable estimates of related quantities, are found to give fairly satisfactory analogous interpretations of the relative heats of hydrogenation of olefins and the equilibria between $\Delta^{\alpha\beta}$- and $\Delta^{\beta\gamma}$-unsaturated carboxylic acids, esters, and nitriles.

Systematic studies of tautomeric equilibria in purely hydrocarbon three-carbon systems have hitherto been restricted to 1 : 3-diarylpropenes and condensed aryl*cyclo*alkyl structures such as indene (see Baker, "Tautomerism," Routledge and Sons, London, 1934, p. 80 *et seq.*). With the object of determining the power of alkyl groups to influence double-bond migrations, we have now examined the equilibria in the simpler examples (I) $\rightleftharpoons$ (II), where R′ = H, R″ = Me, Pr^i, or Bu^t, and R′ = R″ = H or Me.

$$\underset{\text{(I.)}}{Ph{\cdot}CH_2{\cdot}CH{:}CR'R''} \rightleftharpoons \underset{\text{(II.)}}{Ph{\cdot}CH{:}CH{\cdot}CHR'R''}$$

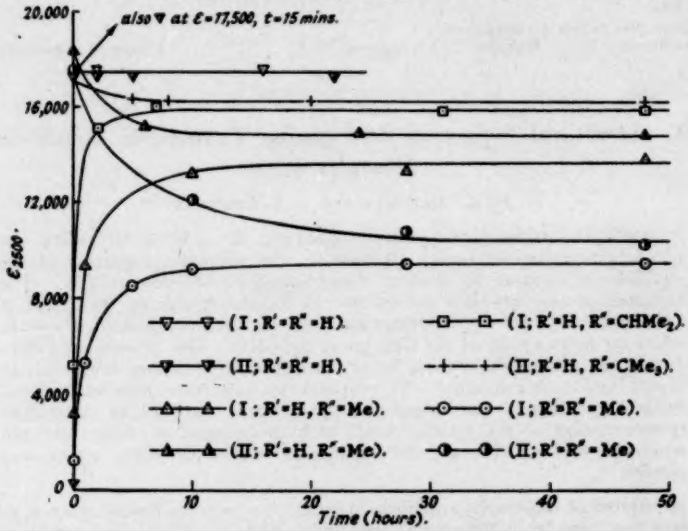
Interconversion of these tautomers occurs readily in methyl-alcoholic alkali at 165° (the half-life periods under the conditions employed being of the order of 1—2 hours), and is conveniently followed quantitatively by estimating the extent of styryl conjugation from ultra-violet absorption measurements. The tautomeric nature of the observed changes has been established by approaching the same equilibrium mixture from hydrocarbons consisting wholly or predominantly of the separate isomers in the cases where R′ = H, R″ = Me; R′ = R″ = H; and R′ = R″ = Me, and by showing in the last system that the components are not otherwise modified or destroyed.

As far as we are aware, these experiments provide the first definite demonstration of the deconjugation of the styryl unit in a mobile prototropic or anionotropic system, and it is therefore of some importance to try to define the responsible structural influences.

In analysing the results shown in Fig. 1, account must be taken of two minor uncertainties. First, a variation is apparent in the value of ε for the several styryl-conjugated isomers. This is undoubtedly due partly to real differences from compound to compound (almost certainly of stereochemical origin) and partly to slight, and variable, contamination with the non-conjugated isomers produced in trivial amounts during their syntheses. For the purpose of deducing the several equilibrium compositions, no significant error is introduced by assuming a uniform value of 17,800, except for propenylbenzene (II; R′ = R″ = H) where the measurements obviously show that no detectable amount of allylbenzene (I; R′ = R″ = H) is present in this system at equilibrium. Secondly, our specimens of (I; R′ = H, R″ = Me) and (I; R′ = R″ = Me) contained about 10% (as estimated from their infra-red spectra) of the 1 : 5-diene-type isomers, $Ph{\cdot}CH_2{\cdot}CH_2{\cdot}CH{:}CH_2$ and $Ph{\cdot}CH_2{\cdot}CH_2{\cdot}CMe{:}CH_2$, respectively. The latter olefins isomerize much more slowly than do the 1 : 4-dienes, and hence the conjugation actually developed will tend to be less than the extent characteristic of the system considered. This factor appears to be responsible for the differences observed in equilibrium composition depending on whether

FIG. 1.

Isomerizations in methyl-alcoholic alkali at 165°.



the 1-phenyl-Δ^1- or 1-phenyl-Δ^2-isomer is examined in these cases, and accordingly we have given more weight to the results obtained from the former isomers.

The equilibrium data and the associated free-energy changes ($-\Delta G = RT \ln K$) summarized in Table I reveal a qualitative correlation between the tautomeric stability of (I) and the number of CH bonds adjacent to the double bond, *i.e.*, first-order hyperconjugation (Mulliken, Riecke, and Brown, *J. Amer. Chem. Soc.*, 1941, **63**, 41) is an effective competing influence opposing the development of styryl conjugation.

The present studies afford in principle an exceptionally sensitive method of defining this relationship quantitatively, but in effect the multiple structural consequences of varying R′ and R″ limit the rigour with which this can be done. First, other electronic processes may contribute to the observed effects: as will be discussed later, substitution as such at a double-bonded carbon atom, irrespective of hyperconjugative properties, exerts a powerful influence in stabilizing compounds such as (I) relative to (II). Secondly, the replacement of a C–H by a C–Alkyl group may alter ΔG by modifying the relative disposition of the neighbouring bonds to an unequal extent in (I) and (II). Thirdly, an uncertain approximation is involved in assuming the total hyperconjugation developed to be strictly proportional to the number of α-methylenic C–H bonds (as, *e.g.*, by Baker *et al.*, *J.*, 1939, 1150; 1940, 692; 1942, 191), owing partly to ignorance concerning the hyperconjugative power of C–C bonds under different conditions.

Finally, the quantitative aspects of cross-hyperconjugation, *e.g.*, >C:CH·C(:CH_2)·CH:C<, are obscure. These features and ambiguities are, however, inherent in all comparable studies of alkyl substitution at the present time, and hence a simplified theory concerned essentially with resultant empirical hyperconjugation changes is of considerable practical value.

Let E_1 denote the resonance energy of conjugation of a phenyl group and a double bond, e_1 that of hyperconjugation between a phenyl group and a C–H bond, e_2 and e_3 that of hyperconjugation between a double bond and a C–H and C–C bond, respectively. If we assume (i) that the resonance energy developed in cross-hyperconjugation is the sum of that developed in the contributing units separately, and (ii) that hyperconjugation involving C–C bonds is negligible except in the absence of C–H bonds, then certain " stabilizing factors " (*s.f.*) can be assigned to the pairs of tautomers (Table II). Equating $s.f._{II} - s.f._{I}$ with $-\Delta G$ yields values for $E_1 - 2e_1$ and e_3 of 2·1 and 0·26 kcal., respectively. It is of interest to examine the validity of these values, and also whether the present analysis in terms of *s.f.*s is of wider applicability.

TABLE I.

Tautomeric system: R′ =	R″ =	$e_{298°}$ at equilibrium.	Proportion of (II) at equilibrium, % (at 165°).	$-\Delta G$ (kcals.).
H	H	17,400	100 *	—
H	CMe_2	16,200	91	2·02
H	$CHMe_2$	15,900	89	1·83
H	Me	14,600	82	1·32
Me	Me	10,200	57	0·25

* See text (p. 2284).

TABLE II.

(I).	*s.f.*	(II).	*s.f.*	$s.f._{II} - s.f._{I}$.
Ph·CH_2·CH:CH·CMe_2 ...	$2e_1 + 2e_2 + 3e_3$	Ph·CH:CH·CH_2·CMe_2 ...	$E_1 + 2e_2$	$E_1 - 2e_1 - 3e_3$
Ph·CH_2·CH:CH·$CHMe_2$ ...	$2e_1 + 3e_3$	Ph·CH:CH·CH_2·$CHMe_2$	$E_1 + 2e_2$	$E_1 - 2e_1 - e_3$
Ph·CH_2·CH:CH·Me	$2e_1 + 5e_3$	Ph·CH:CH·CH_2Me	$E_1 + 2e_2$	$E_1 - 2e_1 - 3e_3$
Ph·CH_2·CH:CMe_2	$2e_1 + 8e_3$	Ph·CH:CH·$CHMe_2$	$E_1 + e_2$	$E_1 - 2e_1 - 7e_3$

Taking $E_1 - 2e_1 = 2{\cdot}1$ kcals., we have $e_3 < 0{\cdot}03$ kcal. There can be little error in assuming $e_1 \sim e_3$, and hence in concluding that $E_1 = 2{\cdot}6$ kcals. This value is less than half that deduced by Pauling and Sherman (*J. Chem. Physics*, 1933, **1**, 606) from heats of combusion (but for amendment see Cottrell and Sutton, *ibid.*, 1947, **15**, 685), but nearly twice that deduced by Dolliver, Evesham, Kistiakowksy, and Vaughan (*J. Amer. Chem. Soc.*, 1937, **59**, 831) from heats of hydrogenation. All these estimates are subject to similar or related ambiguities, and therefore the different data must be analysed equivalently for proper comparison.

In Table III a selection of the extensive data of Kistiakowsky and his collaborators (*loc. cit.*; *J. Amer. Chem. Soc.*, 1935, **57**, 65, 876; 1936, **58**, 137, 146; 1938, **60**, 440) is compared with values of $-\Delta H$ calculated on the assumption that the value for ethylene (*D*) is changed by substitution as follows: Substitution as such at a CH_2 group, irrespective of its nature, introduces a negative $-\Delta H$ term, *S*, which is taken equal to 1·5 kcals. In 1 : 1- or 1 : 2-disubstituted ethylenes, the *S* contribution is doubled, but further substitution is considered to be without effect in this respect. Further negative terms are identified with the resonance energies E_1, e_1 (0·3 kcal.), E_2 (that of the butadienoid unit), e_2 (0·3 kcal.), and E_3 (that of the benzene ring); E_2 and E_3 are determined from examples (11) and (16) as 5·1 and 39·0 kcals., respectively.* Unfortunately, the present technique as applied to the systems studied does not readily allow the equilibria to be determined over a sufficiently wide temperature range to yield accurate values of ΔH. However, comparison of the hydrogenation data with our ΔG values is not seriously unreasonable, since calculations by Kilpatrick, Rosen, Pitzer, and Rossini (*J. Res. Nat. Bur. Stand.*, 1946, **36**, 559) suggest that ΔS differences will not exceed one-quarter of the

* Earlier interpretations of these data emphasise the *cis*-character of the double bonds in the Kekulé formula for benzene, and E_3 has been estimated by the imprecise comparison: 3(−ΔH for *cyclo*hexene)−(−ΔH for benzene). In the present treatment, differences between *cis*- and *trans*-isomers are ignored, partly because no decision can be made as to the extent to which *S* or e_3 or both are effected and partly because our main interest, the value of E_1, is unaffected by this complication. Our tacit assumption that *S* remains the same in the aromatic and aliphatic olefins considered therefore introduces some uncertainty into the value of E_3 deduced, but the fact that $-\Delta H$ calculated for *cyclo*hexene on the above basis (28·4 kcals.) is in good agreement with experiment (28·6 kcals.) suggests that the error is likely to be small.

observed ΔG differences. With this in mind and with recognition that the additive analysis adopted is undoubtedly an over-simplification, the correlation between the observed and calculated values of $-\Delta H$ is regarded as satisfactory, and particularly so that the value of E_1 derived (3·4 kcals.) agrees fairly well with that deduced above.

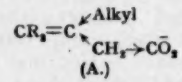
Data more closely comparable with our own are provided by the work of Linstead, Kon, and their collaborators on the equilibria between $\Delta^{\alpha\beta}$- and $\Delta^{\beta\gamma}$-isomers of unsaturated carboxylic acids (in alkaline solution), esters, and nitriles (summarized in Gilman, " Organic Chemistry," Wiley and Sons, 2nd edition, 1944, p. 1042). Some of the extensive results are presented in Table IV, where E_4, E_5, and E_6 are the resonance energies of conjugation of a double bond with a carboxylate ion, carbethoxyl, and nitrile group, respectively, and e_4, e_5, and e_6 are the corresponding hyperconjugation energies. On comparing the conjugating power of these substituent groups with that of an ethylenic double bond, it appears reasonable to take E_4 as 1·5 kcals., $E_5 = E_6$ as 2·0 kcals., and $e_4 = e_5 = e_6$ as 0·2 kcal. To avoid needless repetition and to maintain consistency with Table II, the substitutional factor S is introduced as a *negative s.f.* in compounds containing vinyl groups. The isomeric compositions found and calculated in

TABLE III.

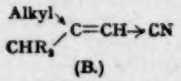
Olefin.	Factors composing $-\Delta H$.	$-\Delta H$ (kcals.) (at 82°). Found.	Calc.
1. $CH_2{:}CH_2$	D	32·58	—
2. $CH_2{:}CHMe$	$D - S - 3e_2$	30·12	30·2
3. $CH_2{:}CH{\cdot}CH_2Me$	$D - S - 2e_2$	30·34	30·5
4. $CH_2{:}CH{\cdot}CHMe_2$	$D - S - e_2$	30·33	30·8
5. $CH_2{:}CMe_2$	$D - 2S - 6e_2$	28·34	27·8
6. $CH_2{:}CMe{\cdot}CH_2Me$	$D - 2S - 5e_2$	28·49	28·1
7. $CH_2{:}CMe{\cdot}CHMe_2$	$D - 2S - 4e_2$	28·00	28·4
8. $CH_2Me{\cdot}CH{:}CHMe$	$D - 2S - 5e_2$	27·95	28·1
9. $CMe_2{:}CHMe$	$D - 2S - 9e_2$	26·92	26·9
10. $CMe_2{:}CMe_2$	$D - 2S - 12e_2$	26·63	26·0
11. $CH_2{:}CH{\cdot}CH{:}CH_2$	$2D - 2S - E_2$	57·07	(57·1) *
12. $CH_2{:}CH{\cdot}CH{:}CHMe$	$2D - 3S - E_2 - 3e_2$	54·11	54·7
13. $CH_2{:}CMe{\cdot}CMe{:}CH_2$	$2D - 4S - E_2 - 6e_2$	53·87	52·3
14. $CH_2{:}CH{\cdot}CH_2{\cdot}CH{:}CH_2$	$2D - 2S - 4e_2$	60·79	61·0
15. $CH_2{:}CH{\cdot}CH_2{\cdot}CH_2{\cdot}CH{:}CH_2$	$2D - 2S - 4e_2$	60·52	61·0
16. PhH	$3(D - 2S) - E_3$	49·80	(49·8) *
17. $Ph{\cdot}CH_2Me$	$3(D - 2S) - E_3 - 2e_2$	48·92	49·2
18. $Ph{\cdot}CH{:}CH_2$	$3(D - 2S) - E_3 + D - S - E_1$	77·48	(77·5) *

* Arbitrary agreement in order to obtain E_2, E_3, and E_1, respectively.

accordance with the principles described above are in good agreement, except in those carboxylates and nitriles where (I) is a β-alkyl-$\Delta^{\beta\gamma}$-compound. With the former substituent the proportion of (I) found is higher than that calculated; with the latter the reverse is true. It is almost certain, therefore, that inductive effects are superimposed upon those hitherto considered: in examples 6, 8, 10, and 11, the electron repulsions of the carboxylate ion and β-alkyl groups reinforce each other to satisfy the inductive electron demand of the double bond in the $\Delta^{\beta\gamma}$-structure (A), whereas they are in opposition in the $\Delta^{\alpha\beta}$-isomer; in example 19, the



(A.)



(B.)

electron attraction of the CN group and repulsion of the β-alkyl group are mutually stimulated by relay through the αβ-double bond (B). Two facts supporting this explanation are (i) the greater influence of a β-ethyl compared with a β-methyl substituent in favouring (A) (cf. 6 with 8 and 10 with 11), and (ii) the absence of similar features in systems containing the less polar carbethoxyl group. These irregularities provide another illustration of what appears to be a general principle, *viz.*, that many systems are potentially responsive to both inductive and hyperconjugative influences, the former coming into prominence in the more polar molecules, the latter in the less (cf. αβ-elimination reactions proceeding in accordance with Hofmann's rule in 'onium compounds, and with Saytzeff's rule in alkyl halides; Dhar, Hughes, Ingold, Mandour, Maw, and Woolf, *J.*, 1948, 2093).

The Structural Factor S.—A special feature of the above interpretations is the emphasis given to S. The importance of this factor in determining the relative stability of vinyl and alkylvinyl isomers is already evident, and is further illustrated by the allylbenzene (I; R′ =

TABLE IV.

(I).	*s.f.*	(II).	*s.f.*	*s.f.* II − *s.f.* I.	−Δ*G* (kcals.).* Found.	Calc.	Equilibrium proportion of (I), %. Found.	Calc.
1. $CH_2{:}CH{\cdot}CH_2{\cdot}CO_2^-$	$2e_2 + 2e_4 - S$	$CHMe{:}CH{\cdot}CO_2^-$	$E_4 + 3e_2$	$E_4 + e_2 - 2e_4 + S$	2·91	2·9	2	2
2. $CHMe{:}CH{\cdot}CH_2{\cdot}CO_2^-$	$5e_2 + 2e_4$	$CH_2Me{\cdot}CH{:}CH{\cdot}CO_2^-$	$E_4 + 2e_2$	$E_4 - 3e_2 - 2e_4$	0·56	0·2	32	43
3. $CH_2Me{\cdot}CH{:}CH{\cdot}CH_2{\cdot}CO_2^-$ ...	$4e_2 + 2e_4$	$CH_2Me{\cdot}CH_2{\cdot}CH{:}CH{\cdot}CO_2^-$	$E_4 + 2e_2$	$E_4 - 2e_2 - 2e_4$	0·78	0·5	26	34
4. $CHMe{:}CH{\cdot}CHMe{\cdot}CO_2^-$	$4e_2 + e_4$	$CH_2Me{\cdot}CH{:}CMe{\cdot}CO_2^-$	$E_4 + 5e_2$	$E_4 + e_2 - e_4$	1·08	1·6	19	10
5. $CH_2Me{\cdot}CH{:}CH{\cdot}CHMe{\cdot}CO_2^-$	$3e_2 + e_4$	$CH_2Me{\cdot}CH_2{\cdot}CH{:}CMe{\cdot}CO_2^-$	$E_4 + 5e_2$	$E_4 + 2e_2 - e_4$	1·56	1·9	11	7
6. $CHMe{:}CMe{\cdot}CH_2{\cdot}CO_2^-$	$8e_2 + 2e_4$	$CH_2Me{\cdot}CMe{:}CH{\cdot}CO_2^-$	$E_4 + 5e_2$	$E_4 - 3e_2 - 2e_4$	−0·36	0·2	62	43
7. $CMe{:}CH{\cdot}CH_2{\cdot}CO_2^-$	$8e_2 + 2e_4$	$CHMe_2{\cdot}CH{:}CH{\cdot}CO_2^-$	$E_4 + e_2$	$E_4 - 7e_2 - 2e_4$	−0·95	−1·0	78	79
8. $CHMe{:}C(CH_2Me){\cdot}CH_2{\cdot}CO_2^-$	$7e_2 + 2e_4$	$CH_2Me{\cdot}C(CH_2Me){:}CH{\cdot}CO_2^-$	$E_4 + 4e_2$	$E_4 - 3e_2 - 2e_4$	−0·99	0·2	79	43
9. $CMe(CH_2Me){:}CH{\cdot}CH_2{\cdot}CO_2^-$	$7e_2 + 2e_4$	$CHMe(CH_2Me){\cdot}CH{:}CH{\cdot}CO_2^-$	$E_4 + e_2$	$E_4 - 6e_2 - 2e_4$	−0·95	−0·7	78	72
10. $CHMe{:}CMe{\cdot}CHMe{\cdot}CO_2^-$	$7e_2 + e_4$	$CH_2Me{\cdot}CMe{:}CMe{\cdot}CO_2^-$	$E_4 + 8e_2$	$E_4 + e_2 - e_4$	0·70	1·6	28	10
11. $CHMe{:}C(CH_2Me){\cdot}CHMe{\cdot}CO_2^-$	$6e_2 + e_4$	$CH_2Me{\cdot}C(CH_2Me){:}CMe{\cdot}CO_2^-$	$E_4 + 7e_2$	$E_4 + e_2 - e_4$	0	1·6	50	10
12. $CH_2Me{\cdot}CH{:}CH{\cdot}CH_2{\cdot}CO_2Et$	$4e_2 + 2e_5$	$CH_2Me{\cdot}CH_2{\cdot}CH{:}CH{\cdot}CO_2Et$	$E_5 + 2e_2$	$E_5 - 2e_2 - 2e_5$	1·45	1·0	8	16
13. $CH_2Me{\cdot}CH{:}CH{\cdot}CHMe{\cdot}CO_2Et$	$3e_2 + e_5$	$CH_2Me{\cdot}CH_2{\cdot}CH{:}CMe{\cdot}CO_2Et$	$E_5 + 5e_2$	$E_5 + 2e_2 - e_5$	1·76	2·4	5	2
14. $CHMe{:}CMe{\cdot}CH_2{\cdot}CO_2Et$	$8e_2 + 2e_5$	$CH_2Me{\cdot}CMe{:}CH{\cdot}CO_2Et$	$E_5 + 5e_2$	$E_5 - 3e_2 - 2e_5$	0·66	0·7	25	24
15. $CMe_2{:}CH{\cdot}CH_2{\cdot}CO_2Et$	$8e_2 + 2e_5$	$CHMe_2{\cdot}CH{:}CH{\cdot}CO_2Et$	$E_5 + e_2$	$E_5 - 7e_2 - 2e_5$	−1·37	−0·5	~90	70
16. $CHMe{:}CMe{\cdot}CHMe{\cdot}CO_2Et$ ...	$7e_2 + e_5$	$CH_2Me{\cdot}CMe{:}CMe{\cdot}CO_2Et$	$E_5 + 8e_2$	$E_5 + e_2 - e_5$	1·57	2·1	5·5	3
17. $CH_2{:}CH{\cdot}CH_2{\cdot}CN$	$2e_2 + 2e_6 - S$	$CHMe{:}CH{\cdot}CN$	$E_6 + 3e_2$	$E_6 + e_2 - 2e_6 + S$	2·3	3·4	~2	0·3
18. $CH_2Me{\cdot}CH{:}CH{\cdot}CH_2{\cdot}CN$	$4e_2 + 2e_6$	$CH_2Me{\cdot}CH_2{\cdot}CH{:}CH{\cdot}CN$	$E_6 + 2e_2$	$E_6 - 2e_2 - 2e_6$	1·08	1·0	14	16
19. $CHMe{:}CMe{\cdot}CH_2{\cdot}CN$	$8e_2 + 2e_6$	$CH_2Me{\cdot}CMe{:}CH{\cdot}CN$	$E_6 + 5e_2$	$E_6 - 3e_2 - 2e_6$	2·7	0·7	~1	24
20. $CMe{:}CH{\cdot}CH_2{\cdot}CN$	$8e_2 + 2e_6$	$CHMe_2{\cdot}CH{:}CH{\cdot}CN$	$E_6 + e_2$	$E_6 - 7e_2 - 2e_6$	−0·79	−0·5	79	70

* At 373° K. for the carboxylates; at 298° K. for the esters and nitriles.

R″ = H)–propenylbenzene (II; R′ = R″ = H) system. The conjugation and hyperconjugation energies being considered as before, but *S* being neglected, the composition of the equilibrium mixture at 165° should be 6 : 94 parts, respectively, in marked disagreement with experiment (Table I). If allowance is made for *S*, the predicted composition becomes 1 : 99. In alkenyl systems, *S* is the dominant factor deciding the equilibrium (III ⇌ IV; X = H)

$$\underset{\text{(III.)}}{CH_2R{\cdot}CHX{\cdot}CH{:}CH_2} \rightleftharpoons \underset{\text{(IV.)}}{CH_2R{\cdot}CH{:}CH{\cdot}CH_2X}$$

which is calculated to be such that 6% of the Δ^1-isomer is present at 165°, in fair agreement with the estimates made by Kilpatrick *et al.* (*loc. cit.*) based mainly on thermochemical data. When certain mobile anionotropic systems are similarly considered, further interesting conclusions emerge. Thus, the fact that the equilibrium mixture of allylic bromides (III ⇌ IV; X = Br) contains approximately 16% of the Δ^1-isomer at 100° (Young, Richards, and Azorlosa, *J. Amer. Chem. Soc.*, 1939, **61**, 3070; cf. Bateman and Cunneen, *J.*, 1950, 941) is now seen to imply that, relatively to a hydrogen atom, a bromine atom attaches itself preferentially at the secondary rather than the primary carbon atom, as expressed quantitatively by a free-energy difference of about 1 kcal. This order of thermodynamic stability, which is consistent with the weakly developed tendency of *n*-alkyl halides to isomerize into *sec.*- and *tert.*-compounds, simulates that found for straight- and branched-chain hydrocarbons (Prosen, Pitzer, and Rossini, *J. Res. Nat. Bur. Stand.*, 1945, **34**, 255, 403), and is of course the reverse of the relative ease of homolytic and heterolytic dissociation.

The origin of *S* is uncertain, but it may well arise from some interaction between the π orbital of the methine carbon atom and the sp^3 orbitals of an adjacent saturated carbon atom, which necessarily has no counterpart when the vinyl substituent is hydrogen. On this view, it would appear to be largely responsible for the effects attributed previously to hyperconjugation (Hughes, Ingold, and McNulty, *J.*, 1940, 899; Dhar *et al.*, *loc. cit.*), although our data indicate that the presence of a carbon atom adjacent to a double bond is much more important as a unique structural feature than the presence of a C–C bond (cf. values of *S* and e_2). This interaction would imply that a vinylic attachment strengthens a C–C more than it does a C–H bond, and therefore that on passing from (III; X = H) to (IV; X = H) *S* measures the change in bonding (exclusive of C–H-bond hyperconjugation) expressed by ($C_{sp^3}C_{sp^3}$—$C_{sp^3}C_{sp^3}$) – (C_{sp^3}H—C_{sp^3}H). Definite independent evidence on this point is non-existent, but Roberts and Skinner's critical evaluation of the relevant thermochemical data (*Trans. Faraday Soc.*, 1949, **45**, 339) reveals just such a difference in the respective dissociation energies in molecules where the parallelism between this quantity and the bond energy is probably very close, *viz.*, $CH_3{\cdot}CH_2$–Me, 82·2; $CH_2{:}CH$–Me, 89·7; $CH_3{\cdot}CH_2$–H, 97·5; $CH_2{:}CH$–H, 102 kcals. g.-mol.$^{-1}$.

Experimental.

Materials.—Allylbenzene (I; R′ = R″ = H) was distilled from sodium, and had b. p. 45·5—46·5°/11 mm., n_D^{18} 1·5122 (Found: C, 91·3; H, 8·6. Calc. for C_9H_{10}: C, 91·5; H, 8·5%). Propenylbenzene (II; R′ = R″ = H) was obtained by isomerization of allylbenzene, and had b. p. 65·0—65·2°/14 mm., n_D^{18} 1·5490.

1-*Phenylbut-2-ene* (I; R′ = H, R″ = Me). Dehydration of 1-phenylbutan-3-ol (from benzylideneacetone) with 50% aqueous sulphuric acid, and fractionation of the product from sodium, yielded this hydrocarbon admixed with *ca.* 19% of the Δ^1- and *ca.* 10% of the Δ^3-isomer. It had b. p. 68—70°/13 mm., n_D^{20} 1·5152 (Found: C, 90·7; H, 9·25. Calc. for $C_{10}H_{12}$: C, 90·9; 9·1%).

1-Phenylbut-1-ene (II; R′ = H, R″ = Me) was prepared (by Dr. D. Barnard) by dehydration of 1-phenylbutan-1-ol (from *n*-propylmagnesium bromide and benzaldehyde; b. p. 73°/0·05 mm.) with anhydrous oxalic acid at 135° (4 hours). It had b. p. 82·0—82·5°/18 mm., n_D^{20} 1·5390 (Found: C, 90·8; H, 9·3. Calc. for $C_{10}H_{12}$: C, 90·9; H, 9·1%).

Isomeric mixture of 4-*methyl*-1-*phenylpent*-2-*ene and its* Δ^1-*isomer* (I and II; R′ = H, R″ = Pr^i). Phenylacetaldehyde (40 g.; freshly distilled) was added dropwise to an ethereal solution of *iso*butylmagnesium bromide prepared from 45 g. of the alkyl halide and 8 g. of magnesium. The resulting 4-*methyl*-1-*phenylpentan*-2-*ol* (18 g.) had b. p. 123°/11 mm., n_D^{19} 1·5050 (Found: C, 81·0; H, 10·25. $C_{12}H_{18}O$ requires C, 80·9; H, 10·1%). Petyunin (*Chem. Abs.*, 1944, **38**, 950) is reported to have prepared this carbinol *via* the Grignard compound of benzyl chloride and *iso*valeraldehyde and to have obtained it as a solid, m. p. 129°. Heating of the carbinol (3 g.) with sodium hydrogen sulphate (1·5 g.) at 160° for 1·5 hours yielded a hydrocarbon mixture (0·5 g.), b. p. 91—92°/10 mm., n_D^{17} 1·5109 (Found: C, 90·1; H, 9·85. Calc. for $C_{12}H_{16}$: C, 90·0; H, 10·0%).

4 : 4-*Dimethyl*-1-*phenylpent*-1-*ene* (II; R′ = H, R″ = Bu^t). Cinnamyl chloride (30 g.) in ether (60 ml.) was added to the Grignard reagent prepared from *tert.*-butyl chloride (48 g.) and magnesium (12 g.). Fractionation of the rather complex product gave a small yield (1 g.) of the required *hydro-*

carbon, b. p. 105—107°/12 mm., n_D^{16} 1·5181 (Found : C, 89·3; H, 10·4. $C_{10}H_{12}$ requires C, 89·7; H, 10·3%).

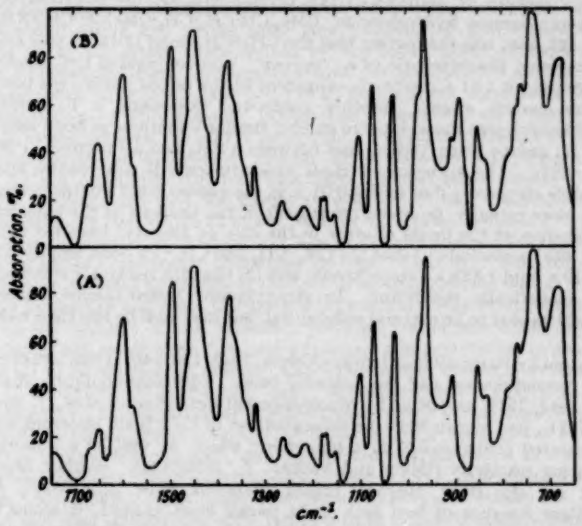
3-Methyl-1-phenylbut-2-ene (I; R′ = R″ = Me) (prepared by Mr. J. A. Lyons). 2-Methyl-4-phenylbutan-2-ol, obtained from benzylacetone (from benzylideneacetone) and methylmagnesium iodide, was dehydrated with 30% aqueous sulphuric acid at 100°. The product had b. p. 81·2—81·6°/11 mm., n_D^{18} 1·5136 (Found : C, 90·5; H, 9·7. Calc. for $C_{11}H_{14}$: C, 90·4; H, 9·6%), and contained *ca.* 10% of 2-methyl-4-phenylbut-1-ene (by infra-red spectroscopic analysis).

3-Methyl-1-phenylbut-1-ene (II; R′ = R″ = Me). 3-Methyl-1-phenylbutan-1-ol, from benzaldehyde and *iso*butylmagnesium bromide, was dehydrated by being heated with anhydrous oxalic acid at 150° for 2 hours. The resulting hydrocarbon had b. p. 81—83°/12 mm., n_D^{18} 1·5331 (Found : C, 90·6; H, 9·5. Calc. for $C_{11}H_{14}$: C, 90·4; H, 9·6%).

Equilibration Procedure.—An accurately weighed quantity (*ca.* 0·1 g.) of the hydrocarbon was dissolved in 10 ml. of methyl-alcoholic potassium hydroxide (20%), and *ca.* 1-ml. portions of the solution were sealed under 10^{-4} mm. pressure in small stout-walled Pyrex tubes. The tubes were heated in a

Fig. 2.

Infra-red absorption spectra. A, *Isomerization product from* (II; R′ = R″ = Me). B, *Synthetic mixture of* (I; R′ = R″ = Me) (43%) *and* (II; R′ = R″ = Me) (57%). *Path lengths*, ~0·05 mm.



bath of boiling 1 : 3-dibromopropane (b. p. 165°/760 mm.) for various times, and the extinction coefficients of the solutions at 2500 A. then measured directly on a Hilger Small Quartz Spectrograph and Spekker Photometer. The results are given in Fig. 1. When the solutions of allyl- and propenyl-benzene were heated for longer than 24 hours, a slight, steady decrease in absorption intensity (at 2500 A.) occurred, possibly owing to polymerization of the former compound leading to effective concentration changes. Blank experiments established that the negligible absorption of the alcoholic alkali was not measurably increased by heating for 60 hours under the isomerization conditions, but among several alcohols and glycols examined methyl alcohol was unique in this respect.

Examination of the Products of an Isomerization Reaction.—3-Methyl-1-phenylbut-1-ene (II; R′ = R″ = Me) (3 g.; ε_{2500} = 17,700) in 20% ethyl-alcoholic potassium hydroxide (12 ml.) was heated in an evacuated, sealed tube at 165° for 50 hours. The recovered hydrocarbon (2·8 g., *i.e.*, 93% recovery) had b. p. 82·0—84·5°/12 mm., n_D^{20} 1·5242, ε_{2500} 10,500 (in methanol) (Found : C, 90·6; H, 9·55. Calc. for $C_{11}H_{14}$: C, 90·4; H, 9·6%), and its infra-red spectrum (Fig. 2) was practically identical with that of a synthetic mixture of the original olefin (57%) and the Δ^2-isomer (43%), except for absorption at 890 cm.$^{-1}$ in the latter arising from the presence of the Δ^3-isomer (2-methyl-1-phenylbut-1-ene).

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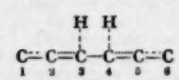
504. *Tautomeric Equilibria in* 1 : 3(4) : 8-*Triene-type Olefins. An Investigation of Double-bond Interaction through Two Methylene Groups.*

By L. Bateman, J. I. Cunneen, and (in part) J. A. Lyons.

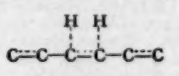
Recent evidence and theories concerned with the existence of some structural peculiarity in 1 : 5-dienes are summarized. An attempt to determine directly whether these dienes are characteristically stabilized by resonance or otherwise is then reported. The experimental basis is to determine the effect on the tautomeric equilibria between 3-substituted 1-phenylprop-1- and -2-enes (see preceding paper) of a double bond, or equivalent unsaturated unit, situated two methylene groups distant from the displaceable Δ^1-double bond. Our results show conclusively that such stabilization is less than is expressed by a free-energy difference (at 165°) of 0·1 kcal.

A remarkable feature of Jeffrey's *X*-ray crystallographic investigation of the molecular structure of geranylamine hydrochloride, $CMe_2{:}CH{\cdot}CH_2{\cdot}CH_2{\cdot}CMe{:}CH{\cdot}CH_2{\cdot}NH_3Cl$ (*Proc. Roy. Soc.*, 1945, *A*, **183**, 388) was the finding that the CH_2–CH_2 bond (1·44 A.) was much shorter than the length considered characteristic of a " normal " non-conjugated C–C single bond (1·54 A.). Lesser contractions (to 1·51 A.) were also apparent in the bonds joining the central CH_2 groups to the methine carbon atoms. Another compound possessing a 1 : 5-diene unsaturation pattern, *viz.*, dibenzyl, was found later to exhibit similar variations of bond length, the distance between the CH_2 groups being 1·48 A., and between a CH_2 and a Ph group, 1·50 A. (*idem, ibid.*, 1947, *A*, **188**, 222). The accuracy of these measurements is not readily specified. Jeffrey estimated possible errors of $\pm 0{\cdot}04$ and $\pm 0{\cdot}01$ A. in the quoted bond lengths in the two structures, respectively. More recently, however, refinement in the analysis of the experimental data and critical consideration of the limits of error in the case of dibenzyl have led Cruickshank (*Acta Cryst.*, 1949, **2**, 65) to conclude (i) that the CH_2–CH_2 and CH_2–Ph bond lengths given above need revision to 1·510 A. and 1·523 A., respectively, and (ii) that the relatively small contractions thus indicated are statistically significant. In geranylamine hydrochloride, therefore, the bond shortening would appear to be real and substantial, but may well be less than half that originally believed.

An explanation by Bateman and Jeffrey (*Nature*, 1943, **152**, 446) of this structural abnormality in terms of hyperconjugation had the following basis. Chemical-equilibria studies (Baker and Hemming, *J.*, 1942, 191), supported by dipole-moment data (Smyth *et al.*, *J. Amer. Chem. Soc.*, 1943, **65**, 89, 1931), had shown that the delocalisation of C–H bond electrons when conjugated with an unsaturated centre exists as a mesomeric effect, as well as a powerful electromeric process influencing reactivity (Baker and Nathan, *J.*, 1935, 1844; Hughes, Ingold, and Taher, *J.*, 1940, 949; see also Dhar, Hughes, Ingold, Mandour, Maw, and Woolf, *J.*, 1948, 2093). Since a 1 : 5-diene consists of two such units joined head-to-head, it seemed reasonable to envisage that the electronic distribution, rather than being as depicted in (I) with the $C_{(3)}$–$C_{(4)}$



(I.)



(II.)

bond insulating the two resonating systems, would tend towards that represented by (II) in order to promote more extensive resonance throughout the carbon chain. This tendency might be expected to develop as the length of the carbon chain increases, and the possible significance in this sense of the finding that the refractivity properties of natural rubber lie between those characteristic of mono-olefins and non-conjugated diolefins on the one hand and conjugated diolefins on the other has been pointed out by Wood and Tilton (Proc. Rubber Tech. Conf., London, 1948, p. 142). Somewhat similar ideas in this or related contexts have been put forward by Szwarc (*Faraday Soc. Discussion*, 1947, **2**, 41), Hinshelwood (*ibid.*, p. 113), Dhar *et al.* (*loc. cit.*, p. 2103), and Dewar (" The Electronic Theory of Organic Chemistry," Oxford, 1949, p. 158). The last author interprets analogously the peculiar alternation in reactivity shown by several series of $\alpha\omega$-disubstituted alkanes—a phenomenon which may also be associated with unusual variations in bond length (Morrison and Robertson, *J.*, 1949, 980, 987, 993, 1001). On the other hand, Walsh (*J.*, 1948, 389) regards the situation in 1 : 5-dienes

quite differently, *viz.*, that the presence of electronegative substituents X in $CH_2X{\cdot}CH_2X$ leads, as a general principle, to a strengthening of the C–C bond. We fail to understand why the CH_2–CH_2 bond rather than the X–CH_2 bond is considered to be mainly affected, for Walsh himself attributes the added bond strength of vinyl compared with alkyl derivatives to just the same cause, and also why electron attraction at *both* ends of a bond should produce a strengthening effect.

Attempts to trace manifestations of the bond shortening in ultra-violet absorption characteristics were first made by Bateman and Koch (*J.*, 1944, 600), who found that geranylamine hydrochloride, in common with hydrocarbon 1 : 5-dienes, absorbs almost identically with comparable mono-olefins in the region 2100—2800 A. This establishes either (i) that the structural abnormality is unique to the crystalline state and does not persist when the molecules are in solution, or (ii) that the light-absorption properties of the 1 : 5-double bonds remain unchanged at the wave-lengths given. The latter is not the remote possibility it might seem on first thoughts because even the spectral consequences of C–H bond hyperconjugation cannot yet be definitely described (cf. Bateman and Koch, *loc. cit.*). Koch (*J.*, 1948, 1123) subsequently extended these investigations to 1 : 6-diphenylhexa-1 : 5-diene (dicinnamyl), where the incorporation of the ethylenic double bonds in styryl units might well enhance the electronic interaction postulated above and at the same time permits the first intense ($N \rightarrow V$ type) absorption band to be accurately characterized by the usual spectrophotometric techniques. Compared with 1-phenylprop-1-ene (propenylbenzene), the absorption maximum of dicinnamyl exhibits a bathochromic shift (from 2470 to 2550 A.) and a slight increase in intensity, which together are suggestive of chromophore interaction. However, even interposition of six saturated carbon atoms between the styryl groups (in 1 : 10-diphenyldeca-1 : 9-diene) produces a similar intensification of absorption though at a reduced wave-length displacement ($\lambda_{max.}$ 2505 A.). Koch concluded therefore that these changes result largely from " weight " or " chain-length " effects and that the extent to which electronic transmission through the saturated carbon atoms is responsible (if at all) remains uncertain. Braude (*J.*, 1949, 1902) has disagreed with this conclusion and argues that Koch's observations actually support his own in demonstrating incomplete electronic insulation by the $-CH_2{\cdot}CH_2-$ unit (of an unspecified nature although valency-bond resonance is thought to be an important factor). The decadiene case is dismissed by postulating some interspatial interaction of the styryl groups. This viewpoint, resting on the validity of this arbitrary postulate, lacks conviction in the absence of supporting evidence, and needs more data and advances in interpretation to gain acceptance.

The only claim to have obtained definite, and quantitative, chemical evidence of the bond contraction in 1 : 5-dienes is by Szwarc (*loc. cit.*). He found that the CH_2–CH_2 bond energy in dibenzyl, calculated by subtracting twice the resonance energy of the benzyl radical (deduced from toluene pyrolysis experiments) from the C–C bond energy in ethane, was 11 kcals. less than that derived from thermochemical data, and interpreted this difference as a measure of bond shortening in good agreement with Jeffrey's data—these imply a difference of 14 kcals. on the basis of Skinner's bond length–bond strength relationship (*Trans. Faraday Soc.*, 1945, **41**, 645). Apart from the fact that comparison with Jeffrey's data is now invalid, we question whether Szwarc's deductions are sufficiently free from uncertainty to make such agreement significant. The interpretative complexities of this type of experiment are well illustrated by a recent study of the pyrolysis of dibenzyl itself (Horrex and Miles, *Faraday Soc. Discussion*, in the press).

The purpose of the present work was to try to devise a direct experimental test of the existence of any unique stabilization of the 1 : 5-diene unit. If shortening of the central C–C bond results from some electronic process involving the flanking ethylenic groups, then a factor exists opposing disruption of the unit and therefore opposing any isomerization involving double-bond displacement. Acyclic terpenoid hydrocarbons of this type are known to be so resistant to such changes (Farmer, *Trans. Faraday Soc.*, 1942, **38**, 358) that quantitative study is greatly impeded, and the occurrence of multiple displacements (cf. the isomerization of hexa-1 : 5-diene to hexa-2 : 4-diene, Faworsky, *J. pr. Chem.*, 1891, **44**, 208) might obscure the data sought. These difficulties can be overcome by arranging that some structural condition fixes one of the double bonds and that the second is a member of a 1 : 4-diene system in which the other member is also fixed. The olefins (III) and (V) possess these features; isomerization in alkaline media at moderate temperatures will therefore be restricted to shifts of the more central ethylenic double bonds to produce the corresponding styryl isomers (IV) and (VI). The interconversion of similar tautomers at 165° is described in the preceding paper, where it is shown that the composition of an equilibrated isomeric mixture affords a sensitive empirical measure of the relative extent of conjugation and C–H bond hyperconjugation in the two isomers. Thus (VII)

and (VIII) co-exist at equilibrium in the proportion 43 : 57 (at 165°). Now (III) and (V) differ from (VII) only in possessing one less α-methylenic hydrogen atom and hence, according to our analysis of the controlling influences (p. 2285), *and if no special energy term intrudes*, the corresponding proportions of (III) to (IV) and (V) to (VI) should be approximately 35 : 65.

$PhCH_2{\cdot}CH{:}CMe{\cdot}CH_2{\cdot}CH_2Ph$ (III.) $PhCH{:}CH{\cdot}CHMe{\cdot}CH_2{\cdot}CH_2Ph$ (IV.)

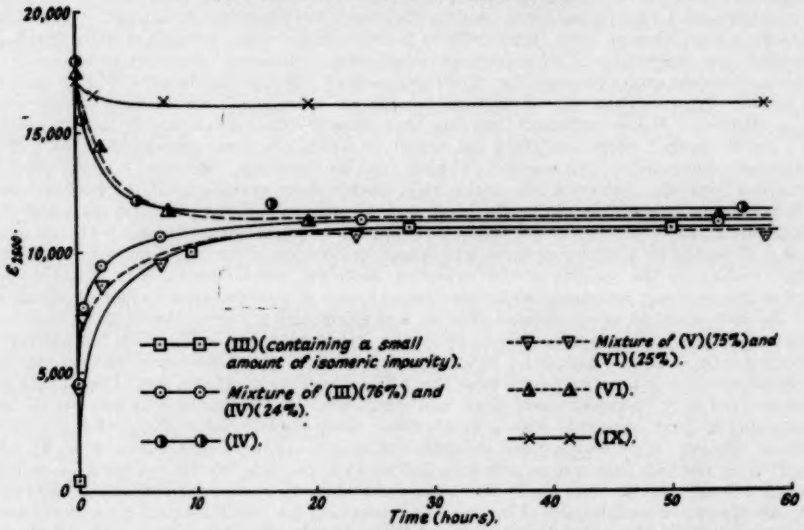
$PhCH_2{\cdot}CH{:}CMe{\cdot}CH_2{\cdot}CH_2{\cdot}CH{:}CMe_2$ (V.) $PhCH{:}CH{\cdot}CHMe{\cdot}CH_2{\cdot}CH_2{\cdot}CH{:}CMe_2$ (VI.)

$PhCH_2{\cdot}CH{:}CMe_2$ (VII.) $PhCH{:}CH{\cdot}CHMe_2$ (VIII.) $PhCH{:}CH{\cdot}CH_2{\cdot}CHMe{\cdot}CH_2{\cdot}CH_2{\cdot}CH{:}CMe_2$ (IX.)

A free energy of only 1 kcal. opposing the destruction of the 1 : 5-diene system would change this ratio to 63 : 37 and 6 kcals.; *i.e.*, Skinner's estimate of the increase in C–C bond strength on contraction from 1·54 to 1·51 A.—if no partly compensating bond lengthening occurs elsewhere—would cause (III) and (V) to be virtually the sole components (99·8%).

The isomerizations observed on heating (III) (containing *ca.* 7% of 3-methylene-1 : 5-diphenylpentane), (IV), and a mixture rich in (III) (containing none of the vinylic isomer) in

Isomerisations in methyl-alcoholic alkali at 165°.



alkaline methyl-alcoholic solution at 165° are shown in the figure; they obviously conform to the same pattern as earlier results (cf. figure, p. 2284). The slightly lower ε at equilibrium observed with the first mixture partly reflects the inertness of the vinylic constituent (cf. p. 2284). To confirm the validity and check the sensitivity of this procedure, (IX) has been similarly examined. This olefin is a substituted *iso*butylstyrene, and actually is found to yield nearly the same equilibrium proportion of its 1-phenyl-Δ^2-isomer (9·5%) as the parent compound (11%; p. 2285). The significant facts disclosed, however, are that the equilibrium mixtures produced from (III) and (IV) and from (V) and (VI) have identical isomeric compositions containing 36% of the non-conjugated forms, and this composition is almost exactly that predicted above on the assumption that the 1 : 5-diene unit is without special effect.

The conclusion from this work is unequivocal: no energy of the magnitude associated with a detectable C–C bond contraction stabilizes chemically the 1 : 5-diene systems examined, and no increase in energy occurs in any individual bond which is not associated with, and almost exactly balanced by, a decrease in another (or others) or with special intermolecular forces. This accords with the near identity of the heat of hydrogenation of hexa-1 : 5-diene with twice that of but-1-ene (Kistiakowsky, Ruhoff, Smith, and Vaughan, *J. Amer. Chem. Soc.*, 1936, **58**,

146) and also with the absence of definite spectroscopic manifestations. It is not inconsistent with the *X*-ray evidence from closely similar systems provided that the qualifying conditions are obeyed. If the bond contraction is real, then the theoretical explanation of Dhar *et al.* (*loc. cit.*; cf. in this connection, Denbigh, *Nature*, 1944, **154**, 642; Wang, *J. Chem. Physics*, 1939, **7**, 1012) is the most acceptable of those hitherto advanced.

Experimental.

3-*Methyl*-1 : 5-*diphenylpent*-2-*ene* (III).—Fractionation of the product obtained on heating 3-methyl-1 : 5-diphenylpentan-3-ol (from dibenzylacetone and methylmagnesium iodide) with aqueous sulphuric acid (30%) at 100° yielded this hydrocarbon (admixed with *ca.* 7% of its 3-methylenepentane isomer), b. p. 126°/0·5 mm., n_D^{14} 1·5605, ε_{2260} 540 (Found : C, 91·3; H, 8·5. Calc. for $C_{18}H_{20}$: C, 91·5; H, 8·5%).

3-*Methyl*-1 : 5-*diphenylpent*-1-*ene* (IV).—3-*Bromo*-1-*phenylbutane*, b. p. 116—118°/14 mm., n_D^{20} 1·5351, was obtained from 1-phenylbutan-3-ol and phosphorus tribromide by reaction first at −10°, then at 100° (Found : C, 56·7; H, 6·3; Br, 37·4. $C_{10}H_{13}Br$ requires C, 56·4; H, 6·1; Br, 37·5%). The Grignard derivative prepared from this bromide (76 g.) and magnesium (9 g.) in ether (250 ml.) was run rapidly on to powdered solid carbon dioxide (300 g.). After treatment of the mixture with ice and hydrochloric acid, and extraction first with ether, then with sodium carbonate solution, followed by acidification of the latter extract, 1-phenylbutane-3-carboxylic acid (25 g.), b. p. 105—107°/0·006 mm., n_D^{20} 1·5113, was obtained (Found : C, 74·3; H, 8·0%; equiv., 179. Calc. for $C_{11}H_{14}O_2$: C, 74·2; H, 7·9%; equiv., 178). By reaction with lithium aluminium hydride in ether, this acid was converted almost quantitatively into 2-methyl-4-phenylbutan-1-ol, b. p. 135°/11 mm., n_D^{18} 1·5173, which gave the corresponding *bromide*, b. p. 129°/14 mm., n_D^{18} 1·5344 (Found : C, 58·2; H, 6·6; Br, 35·2. $C_{11}H_{15}Br$ requires C, 58·2; H, 6·6; Br, 35·3%), with phosphorus tribromide. The Grignard derivative of this bromide (22·7 g.) reacted readily with benzaldehyde (10·7 g.) in ether to give 3-*methyl*-1 : 5-*diphenylpentan*-1-*ol* (17 g.), b. p. 135—136°/0·01 mm., n_D^{18} 1·5506 (Found : C, 85·2; H, 9·0. $C_{18}H_{22}O$ requires C, 85·0; H, 8·7%). Dehydration of this alcohol (3·8 g.) with sodium hydrogen sulphate (1·9 g.) at 160° for 1 hour yielded 3-*methyl*-1 : 5-*diphenylpent*-1-*ene* (2 g.), b. p. 110°/0·01 mm., n_D^{18} 1·5710, ε_{2260} 18000 (Found : C, 91·4; H, 8·6. $C_{18}H_{20}$ requires C, 91·5; H, 8·5%).

A Mixture of (III) *and* (IV).—3-*Methyl*-1 : 5-*diphenylpentan*-2-*ol* (28 g.), b. p. 132—134°/0·005 mm., n_D^{18} 1·5565, was prepared from the Grignard derivative of 3-bromo-1-phenylbutane (79 g.) and phenylacetaldehyde (42 g.) in ether (Found : C, 84·9; H, 8·7%). The olefinic mixture obtained on dehydrating this with sodium hydrogen sulphate under the conditions given above had b. p. 98—100°/0·006 mm. (Found : C, 91·6; H, 8·5. Calc. for $C_{18}H_{20}$: C, 91·5; H, 8·5%). It had ε_{2260} 4800, indicating that 26% of (IV) was present, but it contained no vinylic impurity.

3 : 7-*Dimethyl*-1-*phenylocta*-1 : 6-*diene* (VI).—3 : 7-Dimethyl-1-phenyloct-6-en-1-ol, b. p. 100—102°/0·01 mm., n_D^{18} 1·5132 (Found : C, 83·2; H, 10·3. Calc. for $C_{16}H_{24}O$: C, 82·8; H, 10·4%), prepared from citronellal and phenylmagnesium bromide, was dehydrated by heating with sodium hydrogen sulphate at 160° for 1 hour under nitrogen. Fractionation of the product from sodium gave the required olefin, b. p. 80°/0·01 mm., n_D^{20} 1·5287 (Found : C, 89·7; H, 10·3. Calc. for $C_{16}H_{22}$: C, 89·7; H, 10·3%).

A Mixture of (V) *and* (VI).—2-Methylhept-2-en-6-ol, b. p. 73°/10 mm., n_D^{18} 1·4498, obtained by reducing methylheptenone with lithium aluminium hydride, on reaction with phosphorus tribromide, afforded 6-bromo-2-methylhept-2-ene, b. p. 60°/12 mm., n_D^{18} 1·4711 (Found : C, 50·1; H, 8·0; Br, 41·5. Calc. for $C_8H_{15}Br$: C, 50·2; H, 7·9; Br, 41·9%). A Grignard reaction with this bromide and phenylacetaldehyde gave 3 : 7-*dimethyl*-1-*phenyloct*-6-*en*-2-*ol*, b. p. 97—98°/0·006 mm., n_D^{17} 1·5155 (Found : C, 82·5; H, 10·3%). This was not smoothly dehydrated over oxalic acid and sodium hydrogen sulphate (charring occurred and oxygen-containing compounds were produced), but heating it with boric acid at 160° gave an ester which decomposed at 340° yielding the required mixture, b. p. 75°/0·01 mm. (Found : C, 89·5; H, 10·6. Calc. for $C_{16}H_{22}$: C, 89·7; H, 10·3%). It had ε_{2260} 4340, indicating the presence of 25% of (VI).

4 : 8-*Dimethyl*-1-*phenylnona*-1 : 7-*diene* (IX).—The Grignard derivative of citronellyl bromide (Wagner-Jauregg and Arnold, *Annalen*, 1937, **529**, 274) was treated with benzaldehyde to give 4 : 8-dimethyl-1-phenylnon-7-en-1-ol, b. p. 110°/0·01 mm., n_D^{18} 1·5085 (Found : C, 83·0; H, 10·6. Calc. for $C_{17}H_{26}O$: C, 82·9; H, 10·6%). Dehydration of this alcohol occurred readily over sodium hydrogen sulphate at 160°, the resulting *hydrocarbon* having b. p. 89—90°/0·01 mm., n_D^{18} 1·5250 (Found : C, 89·3; H, 10·7. $C_{17}H_{24}$ requires C, 89·5; H, 10·5%).

Isomerisation Conditions and Procedure.—These were as described in the preceding paper except that the absorptions at 2500 A. were mostly determined on a Hilger Uvispek Photoelectric Spectrometer.

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505. *Synthetic Polypeptides. Part IV.* Preparation and Polymerisation of Oxazolid-2 : 5-diones derived from α-Amino-dicarboxylic Acids.*

By D. Coleman.

An improved method of converting α-amino-dicarboxylic acids into the hydrochlorides of their ω-half-esters has been developed, and applied to the production of several new half-esters of L-glutamic and DL-aspartic acids. Some of these with carbonyl chloride give crystalline oxazolid-2 : 5-diones, including the hitherto undescribed 4-carbomethoxymethyl-DL-oxazolid-2 : 5-dione. Polymers and co-polymers of methyl and ethyl L-glutamate and of methyl DL-aspartate were not of high molecular weight and readily formed gels. This has been ascribed to an intermolecular reaction of terminal amino-groups with ester groups, and the resulting cessation of chain-growth, accompanied by the formation of cross-linkages.

It has been shown (Part III *) that a simple route is available for the preparation of 4-2′-carbethoxyethyl-L-oxazolid-2 : 5-dione by reaction of carbonyl chloride with γ-ethyl glutamate hydrochloride. It has now been found that a modification of the method used in the synthesis of the latter (previously described) results in higher yields and in the isolation of other ω-half-esters of L-glutamic and DL-aspartic acids. The amino-acid is added to the alcohol which contains its equivalent of dry hydrogen chloride (and not the large excess of acid used by most previous workers). The solution obtained after a few minutes' shaking contains a mixture of the hydrochlorides of the amino-acid and of the ω-half-ester. These are readily separated by fractional precipitation with ether. One recrystallisation of the ω-half-ester hydrochloride is usually sufficient. For dissolution of the amino-acids in the acidified alcohols it is absolutely essential that the latter be rigorously anhydrous. The methyl and ethyl glutamate hydrochlorides and methyl DL-aspartate hydrochloride reacted with carbonyl chloride, to give the oxazolid-2 : 5-diones as crystalline compounds. The higher alkyl esters, however, with carbonyl chloride gave oils which were uncrystallisable, doubtless owing to their lower melting points. γ-Ethyl L-glutamate hydrochloride was the only one of the half-esters that was hygroscopic, and in this case it was found convenient to prepare the free ester from the hydrochloride before treatment with carbonyl chloride.

The co-polymers and polymers obtained, such as poly(methyl DL-aspartate) and poly(methyl and ethyl L-glutamate), were of low molecular weight (films could not be cast from dioxan or chloroform). Moreover, the solution when kept for a few days formed irreversible gels. It is probable that the observed gelation was the result of the formation of cross-linkages produced by intermolecular reaction of ester with terminal amino-groups. Loss of amino-groups has been shown quantitatively in the case of poly(benzyl L-glutamate) ester by Hanby and Waley (*J.*, 1950, 3239), who ascribe the disappearance of amino-groups to cyclisation at the chain-ends, with formation of terminal pyrrolidone rings. However, intermolecular reaction probably also occurs, and which reaction predominates will depend on the nature of the solvent [poly(methyl DL-aspartate) will undergo intermolecular reaction only, since it cannot undergo ring closure].

The effect of the solvent on this type of reaction may be illustrated by the methyl esters of glycine and alanine. If these are left in ether or toluene for a few days at room temperature (*i.e.*, under conditions similar to those of the polymerisation) a high yield of the corresponding polypeptides is obtained (Frankel and Katchalski, *J. Amer. Chem. Soc.*, 1942, **64**, 2264), whereas in methanol a similarly high yield but of cyclic products is obtained (diketopiperazines).

The insolubility (and probably, as a consequence, the lack of haptenic activity in biological tests) of synthetic poly-L-glutamic acid contrasted with the solubility of the natural product thus finds a more acceptable explanation than that put forward by Ambrose (*J.*, 1950, 3246). The former is cross-linked and, as is well known, even a small amount of cross-linking is sufficient to modify profoundly the solubility of high polymers (Flory, *J. Amer. Chem. Soc.*, 1941, **63**, 3091). On the other hand, the natural product, although slightly branched, is essentially a linear molecule (Hanby and Rydon, *Biochem. J.*, 1946, **40**, 297) and its solubility is to be expected in view of the irregularities introduced by a slight degree of branching.

Attempts were made to prepare the methyl half-ester of aminomalonic acid as a first step in the preparation of polypeptides containing no side chains but having pendent carboxyl groups.

* Part III, *J.*, 1950, 3222.

Although these do not occur naturally, dipeptides of this type have been prepared (Schneider, *Biochem. Z.*, 1937, **291**, 328).

Experimental.

γ-Ethyl Glutamate Hydrochloride.—L-Glutamic acid (80·0 g.) was added, with shaking, to dry ethanol (330 c.c.) containing hydrogen chloride (24·0 g.); after 20 minutes it had dissolved. Two volumes of ether were added, and after 1 hour the solution was filtered from glutamic acid hydrochloride. More ether (2·5 l.) was added and the crystalline precipitate (m. p. 131°) that separated overnight was recrystallised from ethanol–ether, giving γ-ethyl glutamate hydrochloride (55·0 g.; m. p. 134°). (*Note.* In this and the following experiments it is essential that the esterifying alcohol be rigorously anhydrous. The alcohols in every case were first refluxed for 2 hours with sodium and the corresponding phthalate ester and then distilled just before use.)

Conversion into free ester. The above hydrochloride (5·0 g.) was dissolved in methanol (30·0 c.c.) and cooled to 0°. 10N-Ammonia (2·3 c.c.) was added, and the solution left overnight in the refrigerator, the free ester crystallised (3·0 g.; m. p. 187°). On treatment with carbonyl chloride the corresponding oxazolid-2 : 5-dione was obtained (m. p. 71°).

γ-Methyl Glutamate Hydrochloride.—L-Glutamic acid (18·6 g.) was added to dry methanol (75·0 c.c.) containing the equivalent of hydrogen chloride (4·8 g.). Ether (600 c.c.) was added, and the solution left overnight to crystallise, giving *γ-methyl glutamate hydrochloride* (m. p. 154°; 13·0 g.) (Found: C, 36·25; H, 6·0; N, 7·1; Cl, 17·9. $C_6H_{12}O_4NCl$ requires C, 36·25; H, 6·1; N, 7·1; Cl, 18·3%).

4-2′-Carbomethoxyethyl-L-oxazolid-2 : 5-dione.—γ-Methyl glutamate hydrochloride (8·0 g.), suspended in dioxan (300 c.c.), was treated with carbonyl chloride for 4 hours at 30° and then 1 hour at 40°. A rapid stream of air was passed through the solution for 16 hours to remove excess of carbonyl chloride, and the dioxan was then removed *in vacuo* at 40°. The 4-2′-carbomethoxyethyl-L-oxazolid-2 : 5-dione, recrystallised from chloroform–light petroleum, had m. p. 99° (6·5 g.).

β-Methyl Aspartate Hydrochloride.—Aspartic acid hydrochloride (11·0 g.) was added to dry methanol (70·0 c.c.) containing hydrogen chloride (3·80 g.). After 10 minutes ether (300 c.c.) was added, and the precipitate, recrystallised from ethanol–ether, was *β-methyl aspartate hydrochloride* (m. p. 190°; 8·0 g.) (Found: C, 32·6; H, 5·4; N, 7·6; Cl, 19·4. $C_5H_{10}O_4NCl$ requires C, 32·7; H, 5·45; N, 7·6; Cl, 19·35%).

4-Carbomethoxymethyl-DL-oxazolid-2 : 5-dione.—The foregoing hydrochloride (7·0 g.) in dioxan (250 c.c.) was treated with carbonyl chloride. The *diketone* (5·5 g.), recrystallised from chloroform–light petroleum, had m. p. 84° (Found: C, 41·4; H, 4·6; N, 8·2. $C_6H_7O_4N$ requires C, 41·6; H, 4·5; N, 8·2%).

γ-Propyl L-Glutamate Hydrochloride.—L-Glutamic acid (12·0 g.) was added to *n*-propanol (100 c.c.) containing hydrogen chloride (10·2 g.). After 30 minutes ether (200 c.c.) was added, and the precipitated *hydrochloride*, recrystallised from propanol–ether, had m. p. 152° (6·0 g.) (Found: C, 42·65; H, 7·1; N, 6·3; Cl, 15·7. $C_8H_{16}O_4NCl$ requires C, 42·6; H, 7·1; N, 6·2; Cl, 15·7%).

γ-2-Methoxyethyl Glutamate Hydrochloride.—To L-glutamic acid (14·7 g.), 2-methoxyethanol (60 c.c.), and hydrogen chloride (3·70 g.), was added ether (100 c.c.), which precipitated glutamic acid hydrochloride (6·50 g.). Further addition of ether (100 c.c.) gave *γ-2-methoxyethyl glutamate hydrochloride*, m. p. 174° (7·6 g.). Recrystallised from ethanol–ether, it had m. p. 176° (Found: C, 41·45; H, 6·9; N, 6·1. $C_8H_{16}O_4NCl$ requires C, 41·5; H, 7·0; N, 61·%).

Attempted Preparation of Ethyl Hydrogen Aminomalonate.—(*a*) Aminomalonic acid was shaken with ethanol containing dry hydrogen chloride, but addition of ether or acetone to the solution gave no precipitate of the required half-ester. (*b*) Ethyl aminomalonate was cooled to 0° and ½ equiv. of ethanolic potassium hydroxide (also cooled to 0°) was added with stirring. Methylamine was evolved but no half-ester was isolated.

DL-Aspartic Acid.—After unsuccessful efforts to induce reaction between maleic anhydride and fumaric acid (Tutiya, *Bull. Agric. Chem. Soc. Japan*, 1941, **17**, 87) with ammonia, the method finally adopted (a modification of that of Dunn and Fox, *J. Biol. Chem.*, 1933, **101**, 493) was to treat methyl fumarate (60 g.) with methanol (200 c.c.) containing ammonia (70·0 g.) at 110° for 24 hours in an autoclave. The precipitate obtained (diketopiperazine-diacetamide) was hydrolysed for 6 hours with 6N-sodium hydroxide. After acidification with 6N-hydrochloric and separation of sodium chloride, aspartic acid was isolated by adjusting the pH to 4·0. Recrystallisation from water (300 c.c.) gave DL-aspartic acid (21·0 g.) (Found: N, 10·5. Calc. for $C_4H_7O_4N$: N, 10·5%).

Pyrrolidonecarboxylic Acid.—γ-Ethyl glutamate hydrochloride (9·0 g.) in methanol (63·0 c.c.) was treated with concentrated ammonia solution (10·0 c.c.). After 2 hours the solution was concentrated *in vacuo*, and pyrrolidonecarboxylic acid (4·0 g., m. p. 162°) collected

Polymerisations.—*With sodium salt of phenylalanine as initiator* (see Part III). 0·5-g. samples of the two carbomethoxyalkyloxazolid-2 : 5-diones were allowed to polymerise singly and together in dry pyridine, dioxan, ethyl acetate, or chloroform (7·0 c.c.). The solutions were isolated from atmospheric moisture by means of drying agents contained in a side-tube attached to the reaction vessel as described previously. The solutions rapidly increased in viscosity for the first two days, but within the next few days they invariably formed irreversible gels which did not disperse on the addition of more solvent. When copolymerised in chloroform with the oxazolid-2 : 5-diones of DL-valine, α-amino*iso*butyric acid, or hexylglycine, a similar increase in viscosity culminating in gelation was observed. Thin layers of the solutions when allowed to evaporate did not give coherent films, indicating that the molecular weights were lower than those obtained previously (15,000) with the L-leucine–DL-phenylalanine co-polymer.

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506. *Phyllanthol. A New Alcohol from the Root Bark of* Phyllanthus engleri (*Pax*).

By K. B. Alberman and F. B. Kipping.

The isolation and characterisation of a new triterpene (?) alcohol from *Phyllanthus engleri* (Pax) is described; the name phyllanthol is suggested for it.

The root bark of *Phyllanthus engleri* (Pax) is reported to be used as a criminal poison amongst certain tribes in Rhodesia, and from it Breyer-Brandwijk (*Quart. J. Pharm. Pharmacol*, 1934, **7**, 167) isolated a buff-coloured amorphous glucoside, capable of killing small animals at the extremely low dose-rate of 0·2 mg./kg.; the material was stated to comprise 2% of the dry weight of the bark.

The bark used in the present investigation was supplied by the Colonial Products Research Council and even as received was found to be much less active than reported, as it required 2 g./kg. of the ground bark (equivalent to 40 mg./kg. of the glucoside) to produce fatal results when orally administered to rabbits; also a crude extract prepared by Breyer-Brandwijk's method was fatal only at doses of the order of 100 mg./kg. The remarkable slowness of action observed previously (Breyer-Brandwijk, *loc. cit.*) was confirmed; death usually occurred about 4 days after administration. Post-mortem examination failed to reveal any obvious cause of death.

The difference between our results and those previously obtained may be due to the fact that Breyer-Brandwijk worked in Africa, probably with the fresh bark, whilst our specimen was of necessity not examined until some considerable time after collection. It is also possible that the proportion of glucoside in the bark varies seasonally.

The major constituent of the bark (0·5%) was found to be an alcohol of high melting point which shows no ultra-violet absorption above 210 mμ., thus indicating the absence of a conjugated system, and which contains no reactive double bonds. Analyses of the alcohol and its acetate, benzoate, and *p*-nitrobenzoate suggest a molecular formula $C_{30}H_{50}O$.

The molecular-rotation differences of the alcohol, acetate, and benzoate appear to indicate its triterpenoid nature (Barton, *J.*, 1945, 818) and to relate it to pentacyclic triterpene alcohols, such as ψ-taraxasterol (*idem*, *ibid.*, 1944, 659) to which it shows a distinct similarity.

	M. p. (uncorr.).	$[\alpha]_D$.		M. p. (uncorr.).	$[\alpha]_D$.
ψ-Taraxasterol	218—219°	48°	Alcohol	225—226°	43°
acetate	237—239	53	acetate	259—260	50
benzoate	280—282	72	benzoate	253—254	57

On the basis of the above evidence, the alcohol appears to be new; the name phyllanthol is suggested for it.

Experimental.

M. p.s are corrected. Rotations were measured in chloroform solution in a 1 dm. tube.

Phyllanthol.—The bark (2·3 kg.) was finely ground and then extracted with acetone (10 l.) in a large Soxhlet apparatus for 10 hours. The deep red acetone solution on cooling deposited a colourless amorphous material (6 g.), which was filtered off and exhaustively extracted with light petroleum (b. p. 40—60°). The extract, on evaporation of the solvent, yielded almost pure *phyllanthol* (3·8 g.), m. p. 231—233°. More impure material (9 g.) was obtained by evaporation to dryness of the original acetone solution, followed by extraction with light petroleum. The alcohol was recrystallized from chloroform–light petroleum yielding clusters of tiny needles, m. p. 233—234°, $[\alpha]_D^{15}$ +43° (*c*, 1·23) [Found: C, 83·6, 83·7, 83·1; H, 11·5, 12·0, 12·1%; *M* (Rast), 393, 396. $C_{30}H_{50}O$ requires C, 84·4; H, 11·8%; *M*, 426 (the discrepancy may be due to solvation)].

The alcohol gave a faint yellow colour with tetranitromethane in chloroform, but could not be hydrogenated with hydrogen and platinum and did not react with perbenzoic acid. It did not decolorise a solution of bromine in carbon tetrachloride, or give a precipitate with digitonin; it could not be made to yield a carbonyl derivative with hydroxylamine hydrochloride and sodium acetate. With concentrated sulphuric acid, it gave an orange colour in the cold changing to a reddish-brown on being warmed. With concentrated sulphuric acid and acetic anhydride, it gave a violet colour which faded to a pale red.

Phyllanthol Acetate.—Phyllanthol (0·7 g.) was refluxed with acetic anhydride (3 c.c.) for 1 hour. The crude *acetate* (0·7 g.), which crystallised in glistening plates on cooling, was filtered off and recrystallised from chloroform–methanol giving needles, m. p. 271°, $[\alpha]_D^{14}$ +50° (*c*, 1·54) (Found: C, 82·2, 81·8; H, 11·2, 11·3. $C_{32}H_{52}O_2$ requires C, 82·0; H, 11·1%).

Phyllanthol Benzoate.—Phyllanthol (0·3 g.) and anhydrous pyridine (2 c.c.) were mixed and warmed to about 50°. Benzoyl chloride (0·2 c.c.) was then added and the mixture kept at 50—60° during 2 hours. The crude crystalline *benzoate*, precipitated by the addition of water, was recrystallised from chloroform–methanol giving glistening needles, m. p. 263—264°, $[\alpha]_D^{24}$ +57° (*c*, 0·27) (Found: C, 83·3; H, 10·4. $C_{37}H_{44}O_3$ requires C, 83·7; H, 10·2%).

Phyllanthol p-Nitrobenzoate.—Phyllanthol (0·1 g.), anhydrous pyridine (1·5 c.c.), and *p*-nitrobenzoyl chloride (0·17 g.) were refluxed for 2 minutes, the solution was then diluted with water, and the crude precipitated *ester* recrystallised from chloroform–methanol, giving glistening blades, m. p. 262° (Found: C, 77·0; H, 9·9; N, 2·5. $C_{37}H_{43}O_4N$ requires C, 77·2; H, 9·2; N, 2·4%).

Molecular-rotation Differences.—

	Alcohol (1).	Acetate (2).	Benzoate (3).
$[M]_D$	183°	234°	304°
Δ		$\Delta_{(2-1)}$ 51°	$\Delta_{(3-1)}$ 121°

Acknowledgment is made to Dr. D. H. R. Barton for helpful discussion, to Mr. P. A. Jewell of the Department of Pharmacology, University of Cambridge, for the animal tests, to Mr. E. Milne Redhead of the Royal Botanic Gardens, Kew, for confirming the identity of the starting material, and to the Colonial Products Research Council for a grant (to K. B. A.).

University Chemical Laboratory, Cambridge. [*Received, May 1st*, 1951.]

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507. *The Action of Organo-lithium Compounds on Some Esters and Anhydrides of Dicarboxylic Acids.*

By J. M. Wilson.

The action of organo-lithium compounds on a number of esters and anhydrides of dicarboxylic acids which do not easily react with organo-magnesium compounds has been investigated. Phenyl-lithium reacts satisfactorily with ethyl mesoxalate, methylmalonate, and ethylmalonate, and phthalic anhydride, but not with succinic anhydride, thus demonstrating the superiority of organo-lithium compounds over the usual Grignard reagent. Some glycols not readily prepared by other methods have been synthesised.

Despite the fact that organomagnesium halides react readily with numerous compounds containing more than one carbonyl group (giving as final products *sec.*- and *tert.*-glycols), cases are known where one or more of the carbonyl groups fail to react. Thus, whereas Valeur (*Compt. rend.*, 1901, **132**, 833) found that ethyl oxalate and methylmagnesium iodide readily gave 2 : 3-dimethylbutane-2 : 3-diol, Lamaire (*Bull. Acad. Roy. Belg.*, 1909, 83) failed to obtain the corresponding pentamethyl glycerol by use of ethyl mesoxalate. A similar study of the action of ethylmagnesium bromide gave a mixture of four products, one of which appeared to be 3 : 5-diethyl-3 : 5-dihydroxyheptan-4-one. Lamaire attributed the failure of the medial carbonyl group to react, to the influence of the adjacent groups.

In marked contrast phenyl-lithium (5 mols.) reacts easily and satisfactorily with ethyl mesoxalate (1 mol.), and pentaphenyl glycerol is obtained on hydrolysis.

Organo-metallic derivatives do not as a rule readily give glycols from malonic ester (or its substituted derivatives). According to Dilthey and Last (*Ber.*, 1909, **42**, 2639) the reaction does not proceed to completion, but gives the intermediate hydroxy-ketone: *e.g.*, phenyl-magnesium bromide gave β-hydroxy-ββ-diphenylpropiophenone from ethyl malonate. Wittig and Obermann (*Ber.*, 1934, **67**, 2053) obtained from the intermediate compound the desired glycol, *viz.*, 1 : 1 : 3 : 3-tetraphenylpropane-1 : 3-diol by the use of phenyl-lithium at −80°. The action of Grignard reagents on substituted derivatives of ethyl malonate has been studied by Kalischiev (*J. Russ. Phys. Chem. Soc.*, 1914, **46**, 427), who concluded that the reaction $CR'R''(CO_2Et)_2 + 4MgR'''X$ proceeds to completion only when R′, R″, and R‴ are methyl. This work has now been extended to derivatives of malonic ester of the type $Alk{\cdot}CH(CO_2Et)_2$. The reactions were conducted at −80°. Ethyl methylmalonate (1 mol.) reacts with phenyl-lithium (4 mols.) to give in small yield 2-methyl-1 : 1 : 2 : 3 : 3-pentaphenylpropane-1 : 3-diol, the hydrogen atom attached to the medial carbon atom being replaced by a phenyl group. On the other hand, ethyl ethylmalonate does not react to completion; it gave a large yield of the intermediate β-hydroxy-αββ-triphenylpropiophenone (in this case the alkyl group, and not the hydrogen atom, was replaced by phenyl).

In general, anhydrides of dicarboxylic acids do not readily react with organometallic compounds. Thus Houben and Hann (*Ber.*, 1908, **41**, 1580) found that succinic acid did not react readily with a Grignard reagent. These workers found that succinic anhydride (1 mol.) and phenylmagnesium bromide (6 mols.) in boiling toluene react abnormally, giving in poor yield 1 : 1 : 4 : 4-tetraphenylbutane-1 : 4-diol, *i.e.*, the product normally given by succinic ester. We have found that succinic anhydride and phenyl-lithium in ether or dioxan at −80° fail to give a glycol; the reaction afforded a small yield of diphenyl, and a large amount of a thick viscous oil.

The nature of the reaction between phthalic anhydride and a Grignard reagent is controversial. Bauer (*Ber.*, 1904, **37**, 135) obtained dialkylphthalides by use of alkylmagnesium bromides, and claims to have obtained diphenylphthalide (m. p. 115°) but he did not identify this compound by analysis. On the other hand, Howell (*J. Amer. Chem. Soc.*, 1920, **42**, 2533) claims to have obtained 5-hydroxy-2 : 2 : 5-triphenylbenzofuran by the action of phenylmagnesium bromide. Wittig and Leo (*Ber.*, 1931, **64**, 2395) state phenyl-lithium and phthalic anhydride afford only a resinous compound and triphenylcarbinol. It has now been found that phenyl-lithium gives diphenylphthalide (m. p. 115°). It appears that only one of the CO groups of the anhydride behaves as a true carbonyl group. As phthalic anhydride is sparingly soluble in ether, anhydrous dioxan was used as a solvent. As in the case of succinic anhydride, a large amount of viscous by-product was obtained. This oil has been repeatedly noticed by other workers (cf. Howell, Houben *et al.*, *locc. cit.*), and all efforts to crystallise the compound in its presence fail. However, when the mixture is distilled under reduced pressure, the phthalide distils and may be obtained as a white crystalline compound on recrystallisation from alcohol.

The ethyl mesoxalate used for this work was prepared by the oxidation of ethyl malonate. The action of selenium oxide (Muller, *Ber.*, 1933, **66**, 1668) proved unsatisfactory, whereas an excellent yield was obtained by use of nitrogen oxides (Bouveault and Wohl, *Compt. rend.*, 1903, **137**, 196).

Experimental.

Certain analyses are by Drs. Weiler and Strauss, Oxford. M. p.s are uncorrected.

Reaction of Ethyl Mesoxalate with Phenyl-lithium.—Metallic lithium (4 g.) in a three-necked flask fitted with a mechanical stirrer, reflux condenser, dropping funnel, nitrogen gas inlet, and mercury trap, was covered with anhydrous ether (100 c.c.). Bromobenzene (45 g.) was added and the reaction allowed to proceed for 30 minutes. The solution was allowed to cool, then ethyl mesoxalate (10 g.) in anhydrous ether (100 c.c.) was added slowly. A vigorous reaction ensued, and on its completion the flask was heated gently for 30 minutes. After cooling the product was hydrolysed by ice–water (100 g.). The ethereal layer was separated and the aqueous layer extracted with ether. Ether was removed from the combined ethereal fractions, finally under reduced pressure. The brown semi-solid viscous residue was dissolved in methyl alcohol. Pentaphenyl glycerol was obtained as a white crystalline product after a number of recrystallisations, and had m. p. 163° (yield, 5 g.) (Found : C, 83·2; H, 5·9. Calc. for $C_{33}H_{28}O_3$: C, 83·9; H, 5·9%). It was identified further by the following reaction. The product formed from it (0·3 g.) by acetyl chloride and dimethylaniline in the cold during 5 minutes was treated with a solution of hydroxylamine and the mixture made alkaline with alcoholic potassium hydroxide, heated, and cooled. A reddish-wine colour was produced on acidification and addition of ferric chloride solution.

Reaction of Ethyl Methylmalonate and Ethylmalonate with Phenyl-lithium.—Bromobenzene (62·8 g.), covered with ether (100 c.c.), was treated with lithium (5·6 g.) under nitrogen. On completion of the reaction, an additional 100 c.c. of ether were added, and the solution allowed to cool. The reaction vessel was immersed in a bath containing light petroleum to which solid carbon dioxide was added until the temperature had attained *ca.* −80°. Ethyl methylmalonate (Conrad, *Annalen*, 1870, **204**, 134) (17·4 g.) in ether (100 c.c.) was added gradually. A vigorous reaction ensued, controlled by further addition of solid carbon dioxide to the bath. The product was hydrolysed by ice-water, the ethereal layer separated, and the ether removed by distillation, finally under reduced pressure. The residue was dissolved in alcohol and decolorised with animal charcoal. Repeated crystallisation gave 2-methyl-1 : 1 : 2 : 3 : 3-pentaphenylpropane-1 : 3-diol (2·5 g.), m. p. 155—157° (Found : C, 87·0; H, 6·2. Calc. for $C_{34}H_{30}O_2$: C, 86·8; H, 6·4%).

Reaction of ethyl ethylmalonate (18·8 g. in 100 c.c. of ether) was much less vigorous. The residue obtained after hydrolysis, etc., on purification as above gave β-hydroxy-αββ-triphenylpropiophenone, m. p. 156·5° (10·0 g.) (Found : C, 85·8; H, 5·9. Calc. for $C_{33}H_{28}O_2$: C, 85·4; H, 5·6%).

Reaction of Succinic Anhydride and Phthalic Anhydride with Phenyl-lithium.—A suspension of succinic anhydride (20 g.) in ether (250 c.c.) was added gradually to a warm solution of phenyl-lithium, with gentle heating, and then refluxed for 3 hours. The cooled mixture was then hydrolysed with ice–water (100 c.c.). The ethereal layer was separated and the aqueous layer extracted with ether. The combined extracts were freed from ether, and the brown residue heated under reduced pressure. Crude diphenyl sublimed at 105°/8 mm. It was dissolved in alcohol and was purified by recrystallisation (leaflets, m. p. 67°) (Found : C, 93·1; H, 6·7. Calc. for $C_{12}H_{10}$: C, 93·5; H, 6·5%).

The experiment was repeated with a solution of succinic anhydride (20 g.) in dioxan (150 c.c.) at room temperature and at −80°. In both experiments the only product obtained was a small yield of diphenyl.

Similar experiments were conducted with (*a*) a suspension of phthalic anhydride (20 g.) in ether (300 c.c.) and (*b*) a solution of phthalic anhydride (20 g.) in dioxan (150 c.c.), with gentle heating, and the reactions were completed at the b. p. during 3 hours. The ethereal suspension gave only an unworkable yellowish viscid oil. In contrast, after distillation, at 200°/4 mm., of the hydrolysis product from the dioxan solution a greenish-yellow fluorescent gum was obtained, which readily crystallised from alcohol, to give αα-diphenylphthalide, m. p. 115° (5·0 g.) (Found: C, 82·9; H, 5·15. Calc. for $C_{20}H_{14}O_2$: C, 83·9; H, 4·9%). The m. p. was undepressed when the product was mixed with a specimen prepared by Copisarow's method (*J.*, 1917, **111**, 18).

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MUNICIPAL COLLEGE, PORTSMOUTH. [*Received, March 15th*, 1951.]

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508. *Cordycepin, a Metabolic Product from Cultures of* Cordyceps militaris *(Linn.) Link. Part I. Isolation and Characterisation.*

By K. G. CUNNINGHAM, S. A. HUTCHINSON, WILLIAM MANSON, and F. S. SPRING.

Cordyceps militaris (Linn.) Link has been promoted on various nitrogenous media, yielding solutions which inhibit the growth of many strains of *Bacillus subtilis*. From the culture solutions a crystalline metabolic product, cordycepin, $C_{10}H_{13}O_3N_5$, has been isolated; it appears to be responsible for the total antibacterial activity of the culture solutions.

PREVIOUS work on *Cordyceps militaris* (Linn.) Link has been confined to morphology, life history and relationships (Massee, *Ann. Bot.*, 1895, **9**, 1; Varitchak, *Compt. rend.*, 1927, **184**, 622; Petch, *Trans. Brit. Mycol. Soc.*, 1936, **20**, 216), and physiology (Atkinson, *Bot. Gaz.*, 1894, **19**, 129; Pettit, *Bull. Cornell Univ. Agric. Exp. Station*, 1895, **97**, 339). That the mould might produce an antibiotic was deduced from the observation that the residue of host tissue incorporated in the pseudosclerotum is resistant to decay.

A monoascospore culture from a sporophore collected at Tollymore Park, Co. Down, in the autumn of 1948, was promoted on a peptone (0·5%)–Lemco (0·3%)–glucose (1·0%)–agar (1·5%) medium. The cultures grew vigorously with the development of typical *Cladosporium* conidial stages (Petch, *loc. cit.*) after 5—7 days. The development of antibacterial activity in the liquid medium from a surface culture of the mould was measured by using *B. subtilis*, strain 6752 N.C.T.C., as test organism.

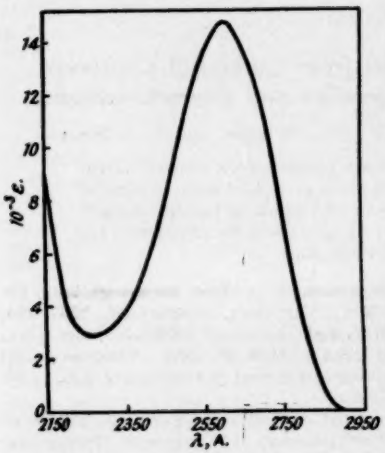
When a peptone (0·5%)–Lemco (0·3%)–glucose (1·0%) medium was used with and without a phosphate buffer, the mould grew vigorously with the production of maximum antibacterial activity after 20—24 days in each case. Similar results were obtained by using a peptone (0·5%)–glucose (1·0%) medium, with and without a phosphate buffer. On the other hand, growth and development of antibacterial activity were very poor when a peptone (0·5%)–Lemco (0·3%) medium was used with a phosphate buffer. Established mycelia maintain their capacity to produce antibacterial activity over a prolonged period on being repeatedly flooded with fresh medium.

The simplest medium which has been found satisfactory for the development of the mould and the production of antibacterial solutions is one containing glucose and a source of amino-acid. In the large-scale production of active culture solutions we employed a medium of the casein hydrolysate "Pronutrin" (0·5%) and glucose (1%). The medium was inoculated from a bulk suspension of conidia; submerged growth was observed on the second day, and was quickly followed by vigorous surface growth and the development of acidity. The medium inhibited the growth of the test strain of *B. subtilis* after 12 days; the first well-defined inhibition, however, was obtained after 20 days; harvesting was commenced on the 28th day. After treatment with charcoal the exhausted medium showed no inhibitory action towards the control strain of *B. subtilis* and was discarded. From the charcoal we isolated a crystalline compound, which we name cordycepin, in yields varying between 25 mg. and 100 mg. per litre of medium.

In aqueous solution, at a concentration between 10 μg. and 100 μg. per ml., cordycepin was found to inhibit the growth of *B. subtilis*, strain 6752 N.C.T.C., in Bouillon broth. Of 45

strains of *B. subtilis* tested, 43 were inhibited in Bouillon broth to varying degrees by cordycepin at a concentration of 1·0 mg. per ml. Cordycepin does not inhibit the development of *Staph. aureus* Oxf. H, *Sar. lutea*, *E. coli*, *B. welchii*, *B. proteus*, *Strep. hæmolyticus*, *S. flexneri*, *Strep. fæcalis*, and *Pasteurella septica*; it inhibits avian-tubercle bacillus at a concentration of 0·1 mg. per ml., Youman's medium being used at 37°, and with Dubos–Tween–albumin medium it inhibits a bovine-type tubercle bacillus at a dilution of 1 : 60,000. The toxicity of cordycepin is of a low order.

Cordycepin crystallises from ethanol and *n*-propanol as transparent needles, from *n*-butanol as lustrous needles or plates, and from water it separates as dull matted needles; in all cases it separates with solvent of crystallisation. A solution of cordycepin in water is nearly neutral (pH 7·1). Analyses of the metabolic product and of its picrate and picrolonate establish the molecular formula, $C_{10}H_{13}O_3N_5$, for cordycepin. A molecular-weight determination by Mrs. Dorothy Hodgkin and Dr. G. J. Pitt, using the *X*-ray crystallographic method, gave the value 247 ± 10, for cordycepin, which is in close agreement with that required for $C_{10}H_{13}O_3N_5$ (251). Cordycepin exhibits an ultra-violet absorption maximum at 2600 A. ($\varepsilon = 14{,}600$) in ethanol (see figure), and is optically active, $[\alpha]_D^{20}$ −47° (in water). The molecule contains three active hydrogen atoms (Zerewitinoff), but contains neither a *C*-methyl group (Kuhn–Roth) nor a methoxyl group (Zeisel); a Herzig–Meyer estimation showed the absence of a *N*-methyl group.



Experimental.

Isolation of Cordycepin.—To a solution of glucose (440 g.) in water (44 l.) was added one of "Pronutrin" (220 g.) in warm water (500 ml.). This medium was dispensed in culture flasks (400 ml. per flask) and sterilised by being autoclaved at 15 lbs./sq. in. for 20 minutes. The cooled flasks were inoculated from a bulk aqueous suspension of conidia of *Cordyceps militaris* (Linn.) Link, which was prepared from a subculture of the mould on peptone (0·5%), glucose (1%), and agar (2%). After incubation at 24° for 28 days, the mixture was filtered, and the filtrate stirred with activated charcoal (500 g.) for 4 days After 24 hours. the supernatant liquor was removed by a siphon. The charcoal was filtered off under suction, air-dried at 24° for 7 days, and extracted (Soxhlet) with acetone for 7 days. The acetone liquor (2 l.) was evaporated, and the residual brown gum washed with cold acetone (20 ml.) and dissolved in hot *n*-butanol (50 ml.). On cooling, the filtered solution deposited a crop of needles (1·1 g.), m. p. 210—220°. Recrystallisation from ethanol gave *cordycepin* as needles, m. p. 225—226°. For analysis a specimen was dried for 6 hours over phosphoric oxide at 78°/0·1 mm. (Found: C, 47·9, 47·8; H, 5·2, 5·2; N, 27·6, 28·3. $C_{10}H_{13}O_3N_5$ requires C, 47·8; H, 5·2; N, 27·9%). Cordycepin crystallises from *n*-propanol as needles, m. p. 225—226°, containing solvent of crystallisation which is difficult to remove. For analysis a specimen was dried for 8 hours over phosphoric oxide at 100°/0·5 mm. (Found: C, 48·1, 48·2; H, 4·9, 5·4%). From water it separates as *hydrated* needles, which were dried for 2 hours at 78°/0·5 mm. (Found: C, 45·3; H, 5·9. $C_{10}H_{13}O_3N_5.H_2O$ requires C, 44·6; H, 5·6%).

Cordycepin Picrate.—To a warm solution of cordycepin (15 mg.) in water (1·0 ml.) was added an excess of saturated aqueous picric acid. The yellow flocculent precipitate was crystallised from water giving *cordycepin picrate* (12 mg.) as needles, m. p. 195° (decomp.) (Found: C, 39·7; H, 3·2; N, 23·4. $C_{10}H_{13}O_3N_5.C_6H_3O_7N_3$ requires C, 40·0; H, 3·4; N, 23·3%).

Cordycepin Picrolonate.—Excess of a saturated aqueous-ethanolic solution of picrolonic acid was added to a warm solution of cordycepin (15 mg.) in water (1·0 ml.). The precipitate of yellow prismatic needles which separated on cooling was collected (10 mg.) and recrystallised from water, yielding *cordycepin picrolonate* as needles, m. p. 240° (decomp.) (Found: C, 46·7; H, 4·2; N, 24·2. $C_{10}H_{13}O_3N_5.C_{10}H_8O_4N_4$ requires C, 46·6; H, 4·1; N, 24·5%).

We are glad to acknowledge our indebtedness to Dr. A. T. Fuller and Dr. P. D'Arcy Hart, of the National Institute for Medical Research, Dr. H. M. Walker and Dr. J. Ungar, of The Glaxo Laboratories Ltd., and Mr. A. A. Berrie for antibacterial tests, and to the Governors of The Royal Technical College, Glasgow, for the award of the Nobel Scholarship (to K. G. C.).

The Royal Technical College, Glasgow.
The University of Glasgow.

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509. *Cordycepin, A Metabolic Product from Cultures of* Cordyceps militaris *(Linn.) Link. Part II.* The Structure of Cordycepin.*

By H. R. Bentley, K. G. Cunningham, and F. S. Spring.

Acid hydrolysis of cordycepin yields adenine and cordycepose. Cordycepose (VI) is shown to be a 3-deoxypentose with a branched carbon chain, and cordycepin is shown to be 9-cordyceposidoadenine (IX).

Part I * described the isolation of a crystalline metabolic product, cordycepin, from culture fluids of *Cordyceps militaris* (Linn.) Link. Analyses of cordycepin and its derivatives indicated a molecular formula $C_{10}H_{13}O_3N_5$ for the former, and preliminary examination revealed that the molecule contains three active hydrogen atoms but no methoxyl, *C*-methyl or *N*-methyl groupings.

The first indication of the nature of cordycepin was obtained from a consideration of its ultra-violet absorption spectrum which exhibits a well-defined maximum at 2600 A. ($\varepsilon \simeq 15{,}000$) in neutral, acid, or alkaline solution. This absorption spectrum is very similar to those of adenine, 9-methyladenine, and adenosine (9-D-ribofuranosidoadenine) (Gulland and Holiday, *J.*, 1936, 768; Table I) but substantially different from that of 7-methyladenine.

Table I.

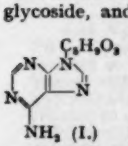
	λ, A.		$\varepsilon_{max.}$	
	N/20-NaOH.	N/20-HCl.	N/20-NaOH.	N/20-HCl.
Cordycepin	2600	2600	14,600	14,400
Adenosine [1]	2600	2600	14,300	14,200
Adenine [1, 2]	2580	2600	13,600	13,200
9-Methyladenine [1]	2600	2600	14,700	14,200
7-Methyladenine [1]	2690	2690	11,400	14,600

[1] Gulland and Holiday (*loc. cit.*). [2] Cf. Loofbourow and Stimson, *J.*, 1940, 844.

This relationship has been confirmed by degradation experiments. Cordycepin dissolves readily in warm, dilute mineral acids to give a solution from which adenine picrate separates on the addition of aqueous picric acid. Furthermore adenine hydrochloride separates rapidly from solutions of cordycepin in concentrated hydrochloric acid, and was identified by analysis and by direct comparison with an authentic specimen in respect of melting point and ultra-violet (Fig. 1) and infra-red absorption spectra (Fig. 2).

Solutions of acid-hydrolysed cordycepin react slowly with 2 : 4-dinitrophenylhydrazine in hydrochloric acid to give a dinitrophenylosazone, $C_{17}H_{16}O_{10}N_8$, which gives the blue-violet coloration with sodium hydroxide in aqueous ethanol characteristic of nitrophenylosazones. By using similar conditions a *p*-nitrophenylosazone, $C_{17}H_{18}O_8N_6$, was obtained which gives a bright blue coloration with the alkali reagent.

Deamination of cordycepin with nitrous acid followed by acid hydrolysis gives hypoxanthine. The isolation of adenine and the nitrophenylosazones shows that cordycepin is an adenine-glycoside, and the deamination of cordycepin to a product from which hypoxanthine was obtained by acid hydrolysis limits the structure of the metabolic product to either a 9- or a 7-glycosidoadenine. The ultra-violet absorption characteristics of cordycepin show that the former is the more likely.



(I.)

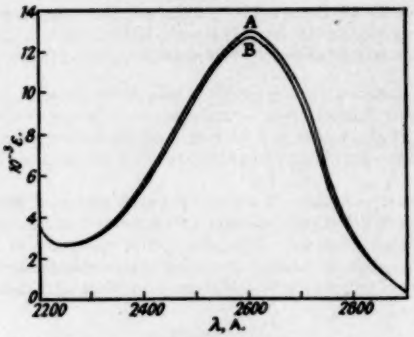
The isolation of a 2 : 4-dinitrophenylosazone, $C_{17}H_{16}O_{10}N_8$, and a *p*-nitrophenylosazone, $C_{17}H_{18}O_8N_6$, from acid-hydrolysed cordycepin corresponds to the formation of a deoxypentose, $C_5H_{10}O_4$. Thus cordycepin is a 9-deoxypentosidoadenine (I). The formation of the osazones, moreover, shows that cordycepin is not a 2-deoxypentoside. Cordycepin is not oxidised by periodate, the conditions described by Lythgoe and Todd (*J.*, 1944, 592) being used; under the same conditions oxidation of adenosine was complete in 4 hours. It follows that cordycepin is a 3-deoxypentoside.

Hydrolysis of cordycepin in dilute hydrochloric acid, followed by the quantitative removal of adenine by means of a cation-exchange resin and then of hydrochloric acid by means of silver carbonate, gave a solution from which an analytically pure sugar, $C_5H_{10}O_4$, has been

* Part I, preceding paper.

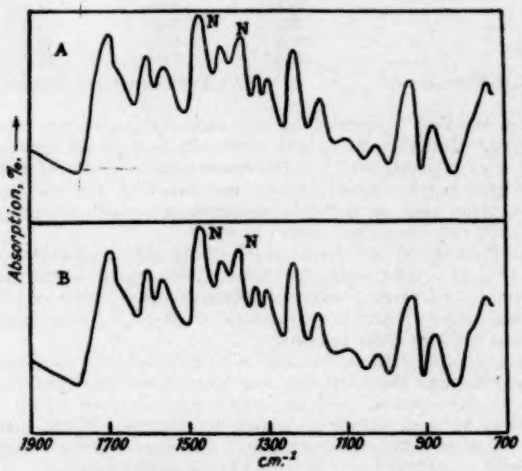
isolated as a pale straw-coloured syrup. The sugar readily reduces Fehling's solution and is oxidised by bromine water to yield a lactone, $C_6H_8O_4$. The latter reacts with phenylhydrazine to yield a crystalline phenylhydrazide, $C_{11}H_{16}O_4N_2$, m. p. 151°, $[\alpha]_D$ +26°. The sugar $C_5H_{10}O_4$ is therefore a 3-deoxyaldopentose.

FIG. 1.



A, *Adenine hydrochloride in ethanol.*
B, *Adenine hydrochloride from cordycepin in ethanol.*

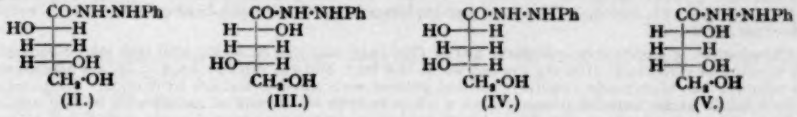
FIG. 2.



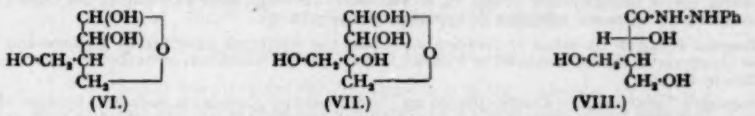
A, *Adenine hydrochloride.*
B, *Adenine hydrochloride from cordycepin* } *in Nujol suspension.*
N, *Nujol absorption bands.*

Of the 3-deoxyaldopentoses, 3-deoxy-L-xylose (3-deoxy-L-ribose) has been prepared by Mukherjee and Todd (*J.*, 1947, 969), and 3-deoxy-D-xylose (3-deoxy-D-ribose) by Kent, Stacey, and Wiggins (*J.*, 1949, 1232). The four phenylhydrazides [(II)—(V)] of the corresponding αγδ-trihydroxyvaleric acids have been described by Nef (*Annalen*, 1910, **376**, 1). These comprise two pairs of enantiomorphs, the D- and the L-*threo*-phenylhydrazide {(II) and (III), m. p. 110°, $[\alpha]_D$ ±26°} and the D- and the L-*erythro*-phenylhydrazide {(IV) and (V), m. p. 150°, $[\alpha]_D$ ±9°}. L-*erythro*-αγδ-Trihydroxyvaleric acid phenylhydrazide (IV) has also been prepared from 3-deoxy-L-xylose by Mukherjee and Todd (*loc. cit.*). The phenylhydrazide,

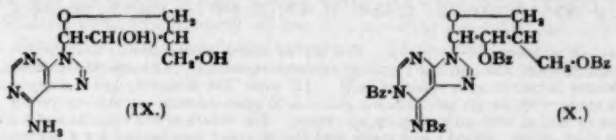
$C_{11}H_{16}O_4N_2$, obtained from the glycosidic fragment of cordycepin is markedly different in melting point from the D- and the L-*threo*-phenylhydrazide (II) and (III), and a comparison of the phenylhydrazide from cordycepin with a specimen of L-*erythro*-$\alpha\gamma\delta$-trihydroxyvaleric acid phenylhydrazide, m. p. 149° $[\alpha]_D$ +4·5°, kindly supplied by Professor A. R. Todd, F.R.S., showed the two isomers to be different. Thus the phenylhydrazide from cordycepin is not identical with any one of the phenylhydrazides (II)—(V).



The sugar obtained by hydrolysis of cordycepin, which we name cordycepose, is not therefore a straight-chain 3-deoxyaldopentose, and the only structure which agrees with its properties is that of a 3-deoxypentose with a branched carbon chain (VI). This represents cordycepose as a relative of apiose (VII), a sugar isolated from parsley (Vongerichten, *Annalen*, 1901, **318**, 121, and subsequent papers; Schmidt, *Annalen*, 1930, **483**, 115).



Accordingly, cordyceponic acid phenylhydrazide is formulated as (VIII); it contains only one asymmetric centre and the phenylhydrazide rule indicates that its dextrorotation requires the configuration shown. In support of the structure (VI) for cordycepose it was found that the *p*-bromophenylosazone of cordycepose, like that of apiose, shows no detectable optical rotation in alcohol. Cordycepin is represented by the structure (IX) in which the configuration of the 2′-centre is known.



Although a crystalline acetate of cordycepin could not be obtained, benzoylation of cordycepin readily gave a crystalline benzoyl derivative, analysis of which indicated that it is a tetrabenzoate and not the expected tribenzoate. The tetrabenzoate is formulated as (X). This behaviour appears to be characteristic of 9-glycosidoadenines since similar benzoylation of adenosine gives a pentabenzoate and not a tetrabenzoate.

Experimental.

Acid Hydrolysis of Cordycepin.—A solution of cordycepin (150 mg.) in hydrochloric acid (2N.; 3·0 ml.) was heated on the steam-bath for 30 minutes. The solution was concentrated under reduced pressure to 1 ml., and the crystalline mass which separated from the cooled solution was collected, washed with ethanol (0·5 ml.), and dried (60 mg., 59%). Recrystallisation from ethanol gave adenine hydrochloride as fine needles, m. p. 285—286° (decomp.) alone or mixed with an authentic specimen (Found, in a sample dried for 3 hours at 78°/0·1 mm.: C, 33·2, 33·4; H, 3·7, 4·1; N, 39·0; Cl, 19·5. Calc. for $C_5H_5N_5,HCl,\frac{1}{2}H_2O$: C, 33·2; H, 3·9; N, 38·8; Cl, 19·6%). A warm solution of the hydrochloride (15 mg.) in water (1·0 ml.) was mixed with a cold saturated aqueous solution of picric acid (2·0 ml.) and the yellow precipitate (25 mg.) was collected, washed with water, and recrystallised from water, giving adenine picrate as long yellow needles, m. p. 290° alone or mixed with an authentic specimen (Found, in a sample dried for 3 hours at 100°/0·1 mm.: C, 36·3, 36·4; H, 2·3, 2·2; N, 30·9. Calc. for $C_5H_5N_5,C_6H_3O_7N_3$: C, 36·3; H, 2·2; N, 30·8%).

Cordycepose 2 : 4-Dinitrophenylosazone.—A solution of cordycepin (400 mg.) in hydrochloric acid (2N.; 5·0 ml.) was heated on the steam-bath for 30 minutes, and the solution cooled and filtered from separated adenine hydrochloride. The filtrate was mixed with a saturated solution of 2 : 4-dinitrophenylhydrazine in hydrochloric acid (2N.; 40 ml.) and set aside at room temperature. Amorphous material which separated during 24 hours was collected and discarded, and precipitates were then collected from the filtrate every day for 10 days. The combined red solid (50 mg.) was washed with

water, dried, and crystallised twice from a small volume of glacial acetic acid whereupon *cordycepose* 2 : 4-*dinitrophenylosazone* was obtained as dark red, microcrystals, m. p. 254—256° (decomp.) (Found : C, 41·6; H, 3·4; N, 22·6. $C_{17}H_{16}O_{10}N_8$ requires C, 41·5; H, 3·3; N, 22·8%). Cordycepose 2 : 4-dinitrophenylosazone gives a blue-violet coloration with sodium hydroxide in ethanol.

Cordycepose p-*Nitrophenylosazone*.—Under similar conditions a solution of cordycepin (100 mg.) in hydrochloric acid (2N.; 2·0 ml.), mixed with a saturated solution of *p*-nitrophenylhydrazine in hydrochloric acid (2N.; 20 ml.), gave *cordycepose* p-*nitrophenylosazone* (50 mg.) which separated from ethanol as a dark-red powder, m. p. 260° (decomp.) (Found : C, 49·8; H, 4·6; N, 20·5. $C_{17}H_{18}O_6N_8$ requires C, 50·7; H, 4·5; N, 20·9%). The *p*-nitrophenylosazone gives a bright blue coloration with sodium hydroxide in ethanol.

Deamination of Cordycepin.—Sodium nitrite (300 mg.) and glacial acetic acid (0·5 ml.) were added to a solution of cordycepin (100 mg.) in hot water (8·0 ml.), and the mixture kept at 70° for 30 minutes. The solution was then made alkaline (brilliant yellow) with aqueous sodium hydroxide. Evaporation of the solution under reduced pressure gave a white residue which was extracted with boiling ethanol (30 ml.). The extract was cooled, filtered from sodium acetate, concentrated, again cooled, and filtered, and finally evaporated to dryness under reduced pressure. The residue was dissolved in hydrochloric acid (*d* 1·18; 2·0 ml.) and heated on the steam-bath for 10 minutes. Evaporation of the solution under reduced pressure gave a dark residue which was extracted with boiling ethanol (25 ml.) (charcoal). The filtered extract was concentrated (10 ml.), and on cooling deposited needles (20 mg.), m. p. 360° (decomp.). A solution of the solid (10 mg.) in hot water (1 ml.) was mixed with a saturated aqueous solution of picric acid and the yellow precipitate was collected, washed with water, and recrystallised from water, giving orange-yellow blades, m. p. 240—250° (decomp., after sintering at 200—220°) alone or mixed with an authentic specimen of hypoxanthine picrate.

Attempted Periodate Oxidation of Cordycepin.—Under the conditions described by Lythgoe and Todd (*loc. cit.*) cordycepin was not oxidised in 3 days; under identical conditions of oxidation adenosine was complete in 4 hours.

Cordycepin Tetrabenzoate.—Cordycepin (50 mg.) was dried over phosphoric oxide for 3 hours at 78°/0·1 mm. and suspended in dry pyridine (3·0 ml.). Benzoyl chloride (1·0 ml.) was added and the mixture shaken to complete solution, set aside for 18 hours at room temperature, and heated on the steam-bath for 5 minutes. The solution was then mixed with crushed ice (20 g.), and the supernatant liquor decanted from a dark red oil which was washed with water (10 ml.) and dissolved in warm ethanol (3·0 ml.). On cooling, the solution deposited a crystalline solid (100 mg.), m. p. 170—175°, which, after recrystallisation from ethanol, gave *cordycepin tetrabenzoate* as lustrous needles, m. p. 179—180° (Found : C, 68·2, 68·5; H, 4·2, 4·2; N, 10·6, 10·5. $C_{38}H_{33}O_7N_5$ requires C, 68·4; H, 4·4; N, 10·5%).

Adenosine Pentabenzoate.—Under similar conditions adenosine (300 mg.) and benzoyl chloride (4·0 ml.) in dry pyridine (5·0 ml.) gave *adenosine pentabenzoate*, which crystallised from aqueous ethanol as needles, m. p. 183—184° (Found : C, 68·6; H, 4·3; N, 8·6. $C_{45}H_{33}O_9N_5$ requires C, 68·6; H, 4·2; N, 8·9%).

Cordycepose.—A solution of cordycepin (3·0 g.) in warm hydrochloric acid (N/10; 100 ml.) was boiled under reflux until the optical rotation became constant. The specific rotation, initial value $[\alpha]_D^{25}$ −38°, became constant at a value of $[\alpha]_D^{20}$ −12° after 270 minutes, and a sample of the solution treated at this stage with saturated aqueous picric acid gave adenine picrate as yellow needles, m. p. 285—290° alone or mixed with an authentic specimen. The solution was neutral and a further addition of hydrochloric acid (N/10; 10 ml.) was made and the mixture was heated for a further 30 minutes to ensure complete hydrolysis. The cooled solution was freed from adenine by passage through a column (30 cm. × 0·5 cm.) of Zeo Carb 215 (Permutit Co., Ltd.) previously washed by decantation and conditioned by treatment alternately with hydrochloric acid (2N.) and ammonia solution (N.) and used in the acid form. The column was washed with water until free from acid, and the total effluent was shown by means of ultra-violet light absorption measurement to contain less than 1 mg. of adenine. The effluent was neutralised with freshly precipitated silver carbonate, filtered, saturated with hydrogen sulphide, and filtered again through charcoal. The clear filtrate was evaporated to dryness under reduced pressure, the residual syrup was dissolved in ethanol (30 ml.), and the solution filtered from a small quantity of inorganic material and evaporated to dryness, finally under high vacuum, giving *cordycepose* as a pale straw-coloured syrup (1·06 g. 66%), $[\alpha]_D^{20}$ −26° (*c*, 0·640 in ethanol) (Found, in a sample dried for 6 hours at 20°/0·1 mm. : C, 44·5; H, 7·9. $C_5H_{10}O_4$ requires C, 44·8; H, 7·5%).

Cordyceponolactone.—Bromine (0·3 ml.) was added to a solution of cordycepose (610 mg.) in water (7·0 ml.), and the mixture was shaken in a closed flask for 60 hours in the dark. After removal of excess of bromine under reduced pressure by means of a stream of air, the solution was diluted (20 ml.) and freed from hydrobromic acid by means of silver carbonate and hydrogen sulphide, as previously described. The solution obtained by filtration through charcoal was evaporated to dryness under reduced pressure and the residual pale brown gum dissolved in ethanol (10 ml.). The solution was filtered from a trace of inorganic material and evaporated to dryness, and the residue heated at 80°/0·1 mm. for 30 minutes, giving *cordyceponolactone* as a pale brown syrup (350 mg., 58%) soluble in aqueous sodium hydrogen carbonate with effervescence, and having $[\alpha]_D^{20}$ +32° (*c*, 0·433 in ethanol) (Found : C, 45·8; H, 6·4. $C_5H_8O_4$ requires C, 45·5; H, 6·1%).

Cordyceponic Acid Phenylhydrazide.—A mixture of cordyceponolactone (55 mg.) and freshly distilled phenylhydrazine (0·1 ml.) in ethanol (0·2 ml.) was heated for 30 minutes under reflux on the steam-bath and then cooled for several hours in the refrigerator. The crystalline product (60 mg., 60%) was collected, a small volume of ethanol being used, and recrystallised from ethyl acetate giving *cordyceponic acid phenylhydrazide* as clustered needles, m. p. 151°, $[\alpha]_D^{20}$ +26° ±3° (*c*, 0·3 in ethanol) (Found : C, 54·8; H, 6·6; N, 11·6. $C_{11}H_{16}O_4N_2$ requires C, 55·0; H, 6·7; N, 11·7%).

Cordycepose p-*Bromophenylosazone*.—A mixture of cordycepose (200 mg.), *p*-bromophenylhydrazine hydrochloride (0·5 g.), and sodium acetate (600 mg.) in acetic acid (15% *v/v*; 5·0 ml.) was heated on the steam-bath for 90 minutes. The discoloured semi-solid product which separated from the cooled mixture was collected and washed free from oily material with the minimum quantity of ice-cold ethanol. The residue (113 mg., 16%) was recrystallised from water (charcoal) giving *cordycepose* p-*bromophenylosazone* as clustered blades, m. p. 163—164°, $[\alpha]_D^{20}$ 0° ±1° (*c*, 0·800 in ethanol) (Found: C, 43·1; H, 3·8; N, 12·0; Br, 35·2. $C_{17}H_{18}O_3N_4Br_2$ requires C, 43·4; H, 3·9; N, 11·9; Br, 34·0%).

The infra-red absorption spectra were determined by Dr. I. A. Brownlie to whom we express our thanks.

Grateful acknowledgment is made to the Governors of the Royal Technical College for the award of the Nobel Scholarship (to K. G. C.).

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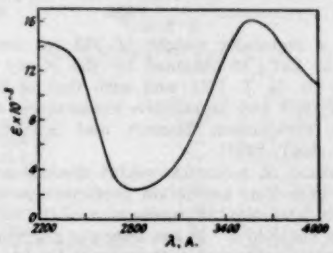
510. *Micro-determination of the Molecular Weights of Picrates by a Spectrophotometric Method.*

By K. G. CUNNINGHAM, WILLIAM DAWSON, and F. S. SPRING.

A method is described for the determination of the molecular weight of a picrate by an examination of its light absorption in the ultra-violet region of the spectrum. The determination is carried out with approximately 2 mg. of the picrate, the accuracy being of the order of ±2%.

ETHANOLIC solutions of picric acid exhibit high-intensity absorption between 2000 and 2500 A., and between 3500 and 4000 A. (figure). Few colourless organic compounds show absorption of appreciable intensity between 3500 and 4000 A. These two facts led us to investigate the possibility of determining molecular weights of bases and hydrocarbons by the measurement of the intensity of light absorption of their picrates within the region 3500—4000 A. By using a Unicam photoelectric spectrophotometer of the compensating-cell type, the light absorption characteristics of a number of picrates were examined. Table I lists the observed intensity of absorption of these picrates at 3800 A., a wave-length chosen from the high-intensity band of



picric acid (3400—4000 A.), at a point remote from the region 2000—3500 A., in which high-intensity absorption is frequently observed in colourless compounds. This choice is to a certain extent arbitrary, similar results to those described being obtained by measurement of the intensity of absorption at other wave-lengths within the high-intensity band of picric acid.

TABLE I.

Picrate.	*c* (mg./100 ml.).	$\log (I_0/I)_{3800}$.	ε_{3800}.
Ethanolamine	1·109	0·512	13,390
Piperidine	1·090	0·469	13,510
Morpholine	1·462	0·620	13,400
2-Aminopyrazine	1·137	0·472	13,450
N-Ethylaniline	1·196	0·459	13,430
Picric Acid	1·107	0·650	13,450

Apart from the weighing operation, the accuracy of the determination depends upon the high sensitivity of the photoelectric spectrophotometer, the value of log (I_0/I) being repeatedly reproducible with an accuracy of $\pm 1{\cdot}0\%$. The average value of ε_{3800} for the five picrates listed in Table I is 13,440, in excellent agreement with the observed value for picric acid. As required by the constant value observed for ε_{3800}, it was found that the bases corresponding to the picrates listed in Table I show no appreciable absorption at 3800 A.

By assuming the average value 13,440 for ε_{3800}, the molecular weights of a number of picrates were determined by the spectrophotometric method, the relationship $M = 13{,}440Cn/\log(I_0/I)$ being used where n is the molar ratio of picric acid : base or picric acid : hydrocarbon in the picrate, and C is measured in g./l. The values obtained for a number of monopicrates, matched quartz cells of 1 cm. thickness being used, are shown in Table II; whole-number atomic weights were employed.

TABLE II.

Picrate.	c (mg./100 ml.).	log $(I_0/I)_{3800}$.	M, found.	M, calc.	Error, %.
Acenaphthene	2·801	0·990	380	383	−0·8
1-Bromonaphthalene	1·353	0·420	433	436	−0·7
2-Methoxynaphthalene	1·161	0·401	389	387	+0·5
2 : 5-Dichloroaniline *	1·421	0·485	394	391	+0·8
Quinoline	1·319	0·491	361	358	+0·8
8-Hydroxyquinoline	1·388	0·498	375	374	+0·3
Adenine	1·361	0·500	366	364	+0·5
2-Methylpyridine	1·165	0·490	320	322	−0·6
1-Methylmorpholine	1·300	0·525	333	330	+0·9
4-Methylglyoxaline	1·959	0·850	310	311	−0·3
Carbazole	1·327	0·443	403	396	+1·8
Cocaine	2·159	0·550	528	532	−0·8
Narcotine	2·938	0·615	642	642	0·0
Strychnine	2·481	0·598	558	563	−0·9
Vomicine	2·530	0·558	609	609	0·0

* 2 : 5-*Dichloroaniline picrate* separates from water as needles, m. p. 86° (Found : C, 37·0; H, 2·3; N, 13·9. $C_6H_5NCl_2,C_6H_3O_7N_3$ requires C, 36·8; H, 2·1; N, 14·3%).

A determination of the molecular weight of cordycepin, a metabolic product isolated from cultures of the mould *Cordyceps militaris* (Linn.) Link, was carried out by using its monopicrate, with the following result :

	c (mg./100 ml.)	log $(I_0/I)_{3800}$	M
Cordycepin picrate	1·036	0·288	483

This value corresponds to a molecular weight of 254 for cordycepin, which is in close agreement both with that of 247 ± 10 obtained by the *X*-ray crystallographic method by Mrs. Dorothy Hodgkin and Dr. G. J. Pitt, and with that of 251 required by $C_{10}H_{13}O_4N_5$ subsequently shown by analytical and degradative examination to be the molecular formula of cordycepin (Cunningham, Hutchinson, Manson, and Spring, *J.*, 1951, 2299; Bentley, Cunningham, and Spring, *J.*, 1951, 2301).

The accuracy of this method of molecular-weight determination is dependent upon the exact determination of the molecular extinction coefficient at the chosen wave-length. A small error in the wave-length determination mechanism of the instrument employed may lead to an appreciable error in the value of ε. It has been our experience that such variations can occur (the value $\varepsilon_{3800} = 13{,}200$ was obtained as the average for 12 picrates examined in a second spectrophotometer similar to the one described above). For this reason it is desirable that the spectrophotometer employed for molecular-weight determination should be standardised against a number of picrates of known molecular weight, matched quartz cells being used in the same aspect.

Grateful acknowledgment is made to the Governors of the Royal Technical College for the award of the Nobel Scholarship (to K. G. C.), and to the Department of Scientific and Industrial Research for the award of a Maintenance Grant (to W. D.).

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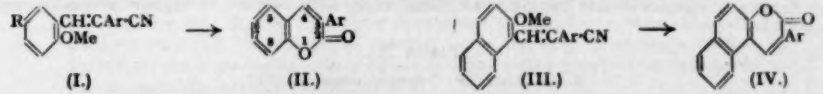
511. *A Synthesis of 3-Arylcoumarins and 3-Aryl-5 : 6-Benzocoumarins.*

By Ng. Ph. Buu-Hoï, Ng. Hoán, and M. R. Khenissi.

A new and convenient procedure is described for the synthesis of diversely substituted 3-arylcoumarins and 3-aryl-5 : 6-benzocoumarins. A large number of these were new compounds, intended for biological study as potential antagonists of sex hormones and as plant-germination inhibitors. The preparation of many new αβ-diarylacrylonitriles is also reported.

It was recently observed (Buu-Hoï, Hoán, and Lavit, *J.*, 1950, 2130; Buu-Hoï and Hoán, *J.*, 1951, 251) that demethylation of β-*o*-methoxyphenyl-α-phenylacrylonitrile and some of its thiophen and thionaphthen analogues by means of pyridine hydrochloride, resulted also in hydrolysis of the nitrile group and lactonisation, with formation of 3-substituted coumarins. The present work is an extension of this procedure for coumarin synthesis to the preparation of a large series of 3-arylcoumarins bearing halogen radicals or hydroxyl groups at various positions. Such compounds were needed for investigations into their possible growth-regulating effects upon plants, and for physiological studies of their potential antagonistic action on sex hormones. It may be recalled that coumarin has been found by various workers (Kuhn, *Naturwiss.*, 1943, **31**, 468; Veldstra and Havinga, *Enzymologia*, 1945, **11**, 373) to inhibit plant germination and the formation of the pollen tube in *Antirrhinum*; in animal experiments, Gley and Mentzer (*Compt. rend. Soc. Biol.*, 1945, **139**, 1055) reported the strong œstrogenic activity of some hydroxy-derivatives of 3-phenylcoumarin, and it is now thought that removal, or replacement by halogen groups, of the hydroxyl radicals would abolish that activity.



(I.) (II.) (III.) (IV.)

The α-aryl-β-*o*-methoxyphenylacrylonitriles (I) used as intermediates were readily obtained by condensation of *o*-methoxybenzaldehyde and 5-bromo-2-methoxybenzaldehyde with variously substituted arylacetonitriles in the presence of alkalis. Similar condensation of

Table I.

Acrylonitriles (I) *and* (III).

Substituent α	Substituent β	M. p.	Formula	Found, % C	Found, % H	Reqd., % C	Reqd., % H
2-Naphthyl	*o*-Methoxyphenyl	104°	$C_{20}H_{15}ON$	84·2	5·0	84·2	4·9
1-Naphthyl	*o*-Methoxyphenyl	132	$C_{20}H_{15}ON$	84·0	5·0	84·2	4·9
p-Fluorophenyl	*o*-Methoxyphenyl	80	$C_{16}H_{12}ONF$	75·6	4·8	75·9	4·7
p-Chlorophenyl	*o*-Methoxyphenyl	116	$C_{16}H_{12}ONCl$	71·1	4·6	71·2	4·4
p-Bromophenyl	*o*-Methoxyphenyl	132	$C_{16}H_{12}ONBr$	61·0	3·9	61·1	3·8
p-Iodophenyl	*o*-Methoxyphenyl	152	$C_{16}H_{12}ONI$	53·0	3·2	53·2	3·3
p-Methoxyphenyl	*o*-Methoxyphenyl	98	$C_{17}H_{15}O_2N$	81·6	6·2	81·9	6·0
p-Tolyl	5-Bromo-2-*o*-methoxyphenyl	100	$C_{17}H_{14}ONBr$	62·0	4·2	62·2	4·3
p-Fluorophenyl	5-Bromo-2-*o*-methoxyphenyl	117	$C_{16}H_{11}ONBrF$	57·5	3·5	57·8	3·3
p-Chlorophenyl	5-Bromo-2-*o*-methoxyphenyl	139	$C_{16}H_{11}ONBrCl$	54·2	3·4	54·5	3·2
p-Bromophenyl	5-Bromo-2-*o*-methoxyphenyl	159	$C_{16}H_{11}ONBr_2$	48·9	2·8	49·0	2·7
p-Iodophenyl	5-Bromo-2-*o*-methoxyphenyl	174	$C_{16}H_{11}ONBrI$	43·4	2·1	43·6	2·3
p-Methoxyphenyl	5-Bromo-2-*o*-methoxyphenyl	150	$C_{17}H_{14}O_2NBr$	59·2	4·6	59·3	4·7
2-Naphthyl	5-Bromo-2-*o*-methoxyphenyl	133	$C_{20}H_{14}ONBr$	65·6	3·9	65·9	3·8
Phenyl	5-Bromo-2-*o*-methoxyphenyl	95	$C_{16}H_{12}ONBr$	61·0	3·8	61·1	3·6
Phenyl	2-Methoxy-1-naphthyl	124	$C_{20}H_{15}ON$	84·0	5·2	84·2	5·3
p-Tolyl	2-Methoxy-1-naphthyl	137	$C_{21}H_{17}ON$	83·9	5·8	84·2	5·7
p-Chlorophenyl	2-Methoxy-1-naphthyl	124	$C_{20}H_{14}ONCl$	74·5	4·3	74·8	4·4
p-Bromophenyl	2-Methoxy-1-naphthyl	135	$C_{20}H_{14}ONBr$	65·7	4·0	65·9	3·8
p-Iodophenyl	2-Methoxy-1-naphthyl	149	$C_{20}H_{14}ONI$	58·1	3·5	58·4	3·4
2-Naphthyl	2-Methoxy-1-naphthyl	149	$C_{24}H_{17}ON$	85·7	5·1	86·0	5·1

2-methoxy-1-naphthaldehyde with arylacetonitriles gave a series of α-aryl-β-(2-methoxy-1-naphthyl)acrylonitriles (III). All these new acrylonitriles are listed in Table I, and the

corresponding 3-arylcoumarins (II) and 3-aryl-5 : 6-benzocoumarins (IV) in Tables II and II*a*, respectively.

The present synthetic route has an advantage, in its simplicity and generality, over those already described for 3-arylcoumarins (von Walther and Wetzlich, *J. pr. Chem.*, 1900, **61**, 178, 194; Funk and von Kostanecki, *Ber.*, 1905, **38**, 939; Meerwein, Büchner, and van Emster, *J. pr. Chem.*, 1939, **152**, 237), and for 3-aryl-5 : 6-benzocoumarins (Bartsch, *Ber.*, 1903, **36**, 1971).

TABLE II.

3-Substituted coumarins (II).

Substituent [a]	M. p.	Formula	Found, % C	Found, % H	Reqd., % C	Reqd., % H
3-β-Naphthyl	174°	$C_{19}H_{12}O_2$	83·5	4·6	83·8	4·4
3-α-Naphthyl	157	$C_{19}H_{12}O_2$	83·8	4·5	83·8	4·4
3-*p*-Fluorophenyl	185	$C_{15}H_9O_2F$	75·1	3·8	75·0	3·7
3-*p*-Chlorophenyl [b]	185	$C_{15}H_9O_2Cl$	—	—	—	—
3-*p*-Bromophenyl	192	$C_{15}H_9O_2Br$	59·5	3·2	59·8	3·0
3-*p*-Iodophenyl	197	$C_{15}H_9O_2I$	51·4	2·6	51·7	2·6
3-*p*-Hydroxyphenyl [c]	202	$C_{15}H_{10}O_3$	75·4	4·4	75·6	4·2
7-Bromo-3-phenyl [d]	196	$C_{15}H_9O_2Br$	57·2	3·1	57·5	2·9
7-Bromo-3-*p*-tolyl	183	$C_{16}H_{11}O_2Br$	60·6	3·6	60·9	3·5
7-Bromo-3-*p*-hydroxyphenyl [c]	230	$C_{15}H_9O_3Br$	56·6	2·9	56·8	2·8
7-Bromo-3-*p*-fluorophenyl [d]	233	$C_{15}H_8O_2BrF$	56·2	2·8	56·4	2·5
7-Bromo-3-*p*-chlorophenyl [d]	235	$C_{15}H_8O_2ClBr$	53·7	2·5	53·7	2·4
7-Bromo-3-*p*-bromophenyl [d]	240	$C_{15}H_8O_2Br_2$	47·1	2·2	47·4	2·1
7-Bromo-3-*p*-iodophenyl [d]	242	$C_{15}H_8O_2BrI$	42·0	2·2	42·2	1·9
7-Bromo-3-β-naphthyl	225	$C_{19}H_{11}O_2Br$	64·9	3·3	65·0	3·1

[a] All these coumarins gave with concentrated sulphuric acid halochromic colours ranging from yellow-green to brown-yellow. [b] Von Walther and Wetzlich (*loc. cit.*) prepared this compound by heating salicylaldehyde with *p*-chlorophenylacetic acid at 300° in a sealed tube and gave m. p. 184°. [c] Soluble in aqueous sodium hydroxide solutions, unlike all the others. [d] Highly sublimable compounds.

TABLE II*a*.

3-Substituted 5 : 6-benzocoumarins (IV).

Substituent [a]	M. p.	Formula	Found, % C	Found, % H	Reqd., % C	Reqd., % H
3-Phenyl [b]	145°	$C_{19}H_{12}O_2$	—	—	—	—
3-*p*-Tolyl	160	$C_{20}H_{14}O_2$	83·1	4·9	83·2	4·9
3-β-Naphthyl	210	$C_{23}H_{14}O_2$	85·4	4·5	85·7	4·3
3-*p*-Chlorophenyl [c]	257	$C_{19}H_{11}O_2Cl$	74·3	3·9	74·4	3·9
3-*p*-Bromophenyl [c]	276	$C_{19}H_{11}O_2Br$	64·6	3·2	64·9	3·1
3-*p*-Iodophenyl [c]	283	$C_{19}H_{11}O_2I$	57·2	2·9	57·3	2·8

[a] All these compounds gave with concentrated sulphuric acid, greenish-yellow to golden-yellow, strongly fluorescent solutions. [b] Bartsch (*loc. cit.*) prepared this compound by heating 2-hydroxy-1-naphthaldehyde with sodium phenylacetate and acetic anhydride, and gave m. p. 142°. [c] Highly sublimable compounds.

None of the coumarins and benzocoumarins reported here has been found to be œstrogenic in the Allen–Doisy test in mice; Gley and Mentzer (*loc. cit.*) reported the activity of 7-hydroxy-3-(*p*-hydroxyphenyl)-4-*n*-propylcoumarin to be only 50 times less than that of œstradiol.

EXPERIMENTAL.

Preparation of Intermediates.—2-Methoxy-1-naphthaldehyde was prepared from neroline by the *N*-methylformanilide reaction (*Org. Synth.*, 1940, **20**, 12), toluene being used in place of the prescribed *o*-dichlorobenzene; 5-bromo-2-methoxybenzaldehyde was obtained by methylation of 5-bromosalicylaldehyde (Auwers and Bürger, *Ber.*, 1904, **37**, 3934) with methyl sulphate in alkaline medium (Wentworth and Brady, *J.*, 1920, **117**, 1043). *p*-Methyl-, *p*-fluoro-, *p*-chloro-, *p*-bromo-, and *p*-iodophenylacetonitrile were prepared by chloromethylation of toluene, *p*-fluoro-, *p*-chloro-, *p*-bromo-, and *p*-iodo-benzene, and treatment of the corresponding chloromethyl compounds with sodium cyanide in ethanol–water. *p*-Methoxyphenylacetonitrile was similarly obtained from the reaction product of *p*-methoxybenzyl alcohol with dry hydrogen chloride. α- and β-Naphthylacetonitrile were prepared from α- and β-methylnaphthalene by side-chain bromination with *N*-bromosuccinimide (Buu-Hoï, *Annalen*, 1944, **556**, 1), and subsequent treatment with sodium cyanide.

Preparation of Acrylonitriles of Types (I) *and* (III).—These were prepared in almost quantitative yield by shaking a mixture of the aldehyde and the arylacetonitrile in warm ethanol with a few drops of 30% aqueous potassium hydroxide. Generally, an oil separated, which readily solidified; the solid was collected, washed with water, and recrystallised from ethanol. The *diarylacrylonitriles* (I) thus

obtained formed long colourless prisms, and their *analogues* of general formula (III) were pale yellow; all gave with concentrated sulphuric acid halochromic colours ranging from yellow to red.

Cyclisation of Acrylonitriles (I) *and* (III) *to Coumarins.*—This was effected by refluxing a mixture of one part of the acrylonitrile and five parts of pyridine hydrochloride until a clear solution was obtained (*ca.* 15 mins.); after cooling, the reaction product was treated with water, and the solid precipitate thus obtained was collected and washed with water, then with methanol. Recrystallisation from benzene or toluene gave the *coumarin* or *benzocoumarin* as well-formed colourless prisms, or shiny needles, resistant to cold alkaline solutions.

The yields obtained in the demethylation-cyclisation reaction were almost quantitative in all cases; those in the acrylonitrile syntheses exceeded 80% except from 1-naphthylacetonitrile, in which case it was probably decreased by steric hindrance.

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512. *Macrozamin. Part II.* The Aliphatic Azoxy Structure of the Aglycone Part.*

By B. W. LANGLEY, B. LYTHGOE, and N. V. RIGGS.

Chemical behaviour of macrozamin is described which shows that the aglycone, an entity apparently incapable of independent existence, contains linked nitrogen atoms; this feature has not previously been met in a natural product. Combined with the results of ultra-violet and infra-red absorption measurements this leads to the formulation of macrozamin as a primeverosyl-oxyazoxymethane (III*a* or III*b*), which has been substantiated by similar absorption measurements on some synthetic aliphatic azoxy-compounds.

A compound prepared by Kijner now shown to be azoxy-*p*-menthane and azoxy*cyclo*hexane, now prepared similarly, are the only compounds so far known in which the carbon atoms adjacent to the azoxy-group carry hydrogen atoms, as in the proposed structure (III).

IN Part I* the carbohydrate component of macrozamin, the toxic constituent of *Macrozamia* spp., was identified as primeverose, and it was shown that the rest of the molecule is uniquely attached to $C_{(1)}$ of the glucose unit through a β-glycosidic link. The present paper describes the structural elucidation of the aglycone part of the molecule, in which the toxicity must reside.

Analysis of the aglycone itself could not be carried out, as it appears incapable of independent existence, but analytical and molecular-weight determinations on macrozamin and its hexa-acetate indicate that the former has the composition $Pv{\cdot}C_5H_8O_2N_2$, where Pv is the primeverosyl residue, $C_5H_9O_4{\cdot}C_6H_{10}O_5$*; this has been confirmed by a study of two stoicheiometric reactions of macrozamin, in which the fates of all the component atoms were determined. It is clear that the aglycone part contains two hydrogen atoms less than a corresponding saturated acyclic structure, and must therefore contain either a double bond or a ring system.

The speed of hydrolysis of macrozamin by dilute acids was that expected for an *O*- or *N*-glycopyranoside; acid concentrations less than 0·1N. or temperatures less than 70° gave an inconveniently slow reaction; with N-hydrochloric acid at 100° hydrolysis was complete within 2 hours. The liberation of the aglycone by hydrolysis, methanolysis, or acetolysis caused it to decompose; after the acidic hydrolysis one of its carbon atoms appeared as formaldehyde (1 mol. of the dimedone derivative was obtained per mol. of macrozamin hydrolysed). The second aglycone carbon atom appeared as methanol, which was shown by conversion into methyl iodide, determined iodometrically. 1 Molar volume of nitrogen gas was also liberated during the hydrolysis. The quantitative formation of xylose and glucose under these conditions having been demonstrated in Part I, the equation for the hydrolysis is

$$C_5H_9O_4{\cdot}C_6H_{10}O_5{\cdot}C_5H_8O_2N_2 + H_2O = C_5H_{10}O_5 + C_6H_{12}O_6 + CH_3{\cdot}OH + CH_2O + N_2 \quad . \quad (1)$$

The quantitative formation of molecular nitrogen in this reaction suggests strongly that macrozamin contains linked nitrogen atoms, a feature not previously met in a natural product.

* Part I, *J.*, 1949, 2716.

The presence of this feature is also indicated by the formation of molecular nitrogen (*ca.* 0·5 mol.) when macrozamin was decomposed by hot dilute sodium hydroxide solution, but this reaction was more complex than the acidic hydrolysis, and other identified products were cyanide ion (*ca.* 0·5 mol.) (cf. Cooper, *Proc. Roy. Soc. New South Wales*, 1940, **74**, 450), formic acid (*ca.* 0·5 mol.), and traces of methylamine and ammonia. Even weak alkalis sufficed to decompose macrozamin; the primeverose was liberated intact by the action of cold N-aqueous ammonia; this behaviour is noteworthy because relatively few alkali-labile glycosides are known.

The most direct evidence of the presence of linked nitrogen atoms in macrozamin was obtained by treating its hexa-acetate, dissolved in chloroform–ether, with dry hydrogen chloride, whereupon hydrazine dihydrochloride was precipitated in a yield of *ca.* 75%. Formaldehyde (*ca.* 0·8 mol.) was also produced in this reaction, but the sugar fragment could not be obtained pure; probably it was first set free as the hexa-acetate with an unprotected glycosidic centre and then decomposed by the vigorous conditions. The results of this reaction made it likely that the linked nitrogen atoms of macrozamin occupy an oxidation level higher than that of hydrazine, and underwent reduction to this level by an oxidation–reduction similar to that in which bis-ω-nitrosophenylmethane is converted into benzoylhydrazine by dry hydrogen chloride (Behrens, *Annalen*, 1902, **323**, 272).

The nitrogen atoms of macrozamin have no basic properties; its aqueous solutions are neutral and its hexa-acetate forms neither salts with acids nor a quaternary compound with methyl iodide. This affords evidence of the presence of linked nitrogen atoms which, unlike the chemical evidence presented, is not invalidated by the possibility of molecular rearrangement. To lose its basic character a nitrogen atom must be (*a*) triply linked, as in nitriles, (*b*) conjugated with a group like C=O, (*c*) doubly linked to a carbon and singly to an electronegative atom, like oxygen, as in oximes, or (*d*) have at least two valencies attached to oxygen or nitrogen. Spectroscopic results described below show that the first three conditions do not apply to macrozamin, so the fourth must be fulfilled in respect of both nitrogen atoms. This can only be done if they are linked together, so that each assists in weakening the basic character of the other.

Unlike most compounds containing linked nitrogen atoms, macrozamin did not give a hydrazine derivative when reduced. In neutral solution, hydrogen and palladium caused no reduction, and hydrogen and platinum only a partial reduction, after which much macrozamin was recovered unchanged. For success an acid seemed necessary, and the course of the reduction depended then both on the acid strength and the reagent used. In cold 5N-hydrochloric acid, stannous chloride caused the reduction

$$\mathrm{Pv{\cdot}C_5H_9O_4N_3 + 3SnCl_2 + 6HCl = PvOH + 3SnCl_4 + CH_3{\cdot}NH_2 + NH_3 + CH_2O} \quad . \quad . \quad (2)$$

This is the second of the two stoicheiometric reactions mentioned above; its quantitative nature was confirmed with respect to stannous chloride by iodometric titration, to total volatile base by distillation and titration with acid, to methylamine by a modification of Cromwell's method (*Biochem. J.*, 1949, **45**, 84), and to formaldehyde by weighing of the dimedone derivative. The formation of reducing sugar was shown qualitatively. When macrozamin was reduced with platinum and hydrogen in 0·2N-hydrochloric acid, 4 mols. of hydrogen were absorbed, the sugar was liberated as primeverose, and 2 mols. of a volatile base consisting essentially of methylamine were formed; this corresponds to a reaction

$$\mathrm{Pv{\cdot}C_5H_9O_4N_3 + 4H_2 = PvOH + 2CH_3{\cdot}NH_2 + H_2O} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

Similar reduction in the presence of N-acid followed a mixed course in which reactions of equations (2) and (3) appeared to proceed simultaneously, and a similar mixed reaction was obtained with zinc dust and acetic acid. Sodium amalgam could not be used because of the alkali-lability of macrozamin.

It is surprising that no hydrazine derivatives were formed in these experiments; in only a few compounds containing linked nitrogen atoms is the N–N link split in a similar way, *e.g.* in *ON*-dimethyl-*N*-nitrosohydroxylamine (I) (Boese, Jones, and Major, *J. Amer. Chem. Soc.*, 1931, **53**, 3530). The reduction of macrozamin expressed by equation (2) shows that there is a link between one nitrogen and one carbon atom of the aglycone. There is probably also a link between the second nitrogen and the second carbon atom, as shown by the formation of 2 mols. of methylamine in equation (3), but some caution is necessary about this conclusion, as it is possible, though not probable, that the second molecule of methylamine might arise from reductive condensation of ammonia and formaldehyde produced as in equation (2).

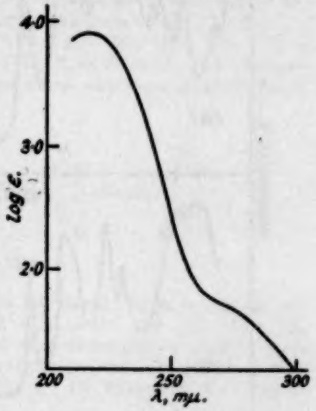
The reactions described are the only useful ones found for macrozamin. The latter was unaffected by nitrous acid and did not respond to tests for a peroxide link. Its hexa-acetate was not changed by treatment with bromine in chloroform, ethereal diazomethane, chromic acid in acetic acid, or perbenzoic acid in chloroform. Although at this stage of the work a possible structure could be suggested (cf. Lythgoe and Riggs, *Experientia*, 1949, **5**, 471), the only conclusions rigidly established were that (*a*) the aglycone contains the system C–N–N and (*b*) the primeverose is linked to it through oxygen or nitrogen. It was clear that wider conclusions could be drawn if it could be settled whether the aglycone contained a double bond. This point was decided spectroscopically.

The ultra-violet extinction curve of macrozamin (Fig. 1) is a single band characterised by a maximum at 217 mμ. and an inflexion at *ca.* 275 mμ.; it was clear from this curve that macrozamin is covalently unsaturated. No chromophoric group showing the same kind of absorption has so far been described, but certain features of similarity are present in the ultra-violet absorption spectra of primary and secondary nitramines (R. N. Jones and Thorn, *Canadian J. Res.*, 1949, **27**, 828), and of certain *C*-nitro-compounds and their *aci*-forms (Kortüm, *Z. physikal. Chem.*, 1939, **43**, *B*, 271).

Fig. 2 reproduces part of the infra-red absorption spectra of macrozamin and its hexa-acetate. That of macrozamin shows no bands between 1600 and 1800 cm.$^{-1}$, showing that double bonds involving carbon are absent from the molecule; the band at *ca.* 1760 cm.$^{-1}$ in the spectrum of the hexa-acetate is due to the ester carbonyl groups. Characteristic of both spectra is the strong band near 1530 cm.$^{-1}$, which is not shown by hepta-acetyl primeverose, and was apparently due to an unsaturated centre in the aglycone of a kind not previously studied by infra-red spectral methods. Relatively few groups are known which cause absorption in this region; among them are the C–NO_2 group (1540—1590 cm.$^{-1}$) and the N–NO_2 group of certain nitramines (1500—1590 cm.$^{-1}$) (R. N. Jones, personal communication).

FIG. 1.

Light absorption of macrozamin in water.



The acidic hydrolysis of macrozamin (equation 1) could now be analysed to determine the oxidation level of the linked nitrogen atoms. If no oxidation–reduction were involved this level would be that of molecular nitrogen; but hydrolyses of organic compounds in which nitrogen is liberated frequently proceed by oxidation–reduction, in which the nitrogen system may either become oxidised during the hydrolysis, as with formamylazo*iso*butyronitrile (Thiele and Stange, *Annalen*, 1894, **283**, 34), or reduced, as with *ON*-dimethyl-*iso*nitramine (II) (Franchimont and Umbgrove, *Rec. Trav. chim.*, 1896, **15**, 213). In the present case it was therefore necessary to consider the adjacent lower (di-imide) and higher (nitrous oxide) oxidation levels as well as that of molecular nitrogen. The first of these possibilities would imply that macrozamin is an azo-compound, and can be discarded because these compounds show two ultra-violet absorption bands at about 220 and 340 mμ. (Kortüm and Finckh, *Z. physikal. Chem.*, 1940, **45**, *B*, 32; Kortüm, *ibid.*, 1941, **50**, *B*, 361). The possibility that macrozamin is a representative of the higher (nitrous oxide) level must also be discarded, although attractive at first sight, because similar representatives, such as (I) and (II), are isomeric with the hypothetical aglycone of macrozamin and when hydrolysed give the same products as the latter; all such representatives, if they contain a double bond, as established for macrozamin, provide no position for the glycosidic attachment of the primeverosyl group to oxygen or nitrogen. The level of the nitrogen system of macrozamin is therefore that of molecular nitrogen, a level which is represented by diazo-compounds, azo-ethers, nitrosamines, and azoxy-compounds. A diazo-structure is excluded by the stability towards cold dilute acids, and an azo-ether structure by the relatively high thermostability of macrozamin [the known azo-ethers decompose explosively at room temperature (Bamberger, *Ber.*, 1895, **28**, 225)]. A nitrosamine structure is excluded because nitrosamines show two absorption bands in the ultra-violet near 235 and 340 mμ. (Goldberg and Kirchenstein, *Helv. Chim. Acta*, 1943, **26**, 288; R. N. Jones and Thorn, *loc. cit.*). Macrozamin must therefore be an aliphatic azoxy-compound, namely, a primeverosyl-

oxyazoxymethane (III*a* or III*b*); the evidence is inadequate to decide between the two alternatives.

Pv = $C_5H_9O_4$·$C_6H_{10}O_5$·

Very few aliphatic azoxy-compounds have been obtained until now; little is known of their chemical, and nothing of their spectroscopic, properties. The *s*-dinitrotetramethylazoxymethane described by Scholl and Born (*Ber.*, 1895, **28**, 1366) and Wieland's azoxyformic acid bisamidoxime (*Ber.*, 1905, **38**, 1452), are probably genuine, but the " bisazoxymethane "

FIG. 2.

Infra-red absorption spectra of (A) macrozamin and (B) hexa-acetyl macrozamin.

(Samples were examined as pastes with Nujol, to which the bands near 6·85 and 7·25μ. are due.)

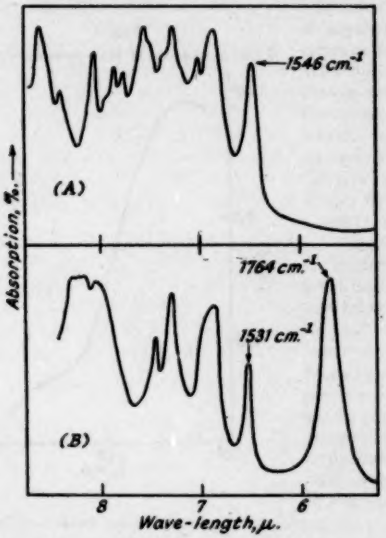
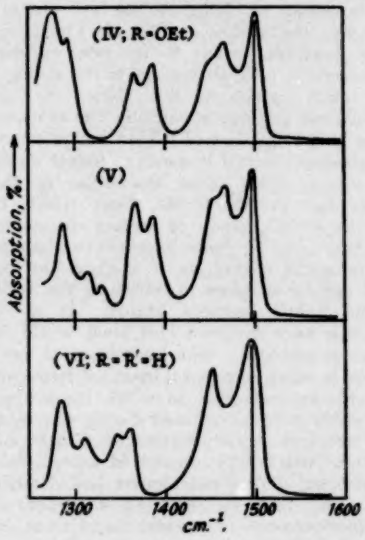
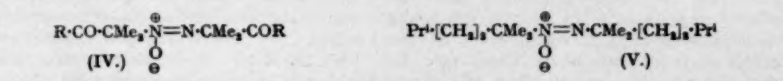


FIG. 3.

Infra-red absorption of ethyl 2-azoxyisobutyrate (IV; R = OEt), *2-azoxy-2 : 5-dimethylhexane* (V) *and azoxycyclohexane* (VI; R = R′ = H) *(liquid films).*

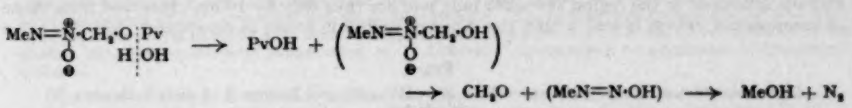


described by Hantzsch and Lehmann (*Ber.*, 1900, **33**, 3669) was shown by Curtius and his colleagues (*Ber.*, 1908, **41**, 3161) to be 1 : 2 : 4 : 5-tetrazine; and the nature of Bamberger's " bisazoxybenzyl " (*Ber.*, 1897, **30**, 2281) has never been clarified. The only well-authenticated aliphatic azoxy-compounds are those described by Aston and his collaborators; (IV; R = Me) and (IV; R = OEt) were obtained (*J. Amer. Chem. Soc.*, 1932, **54**, 1530; 1934, **56**, 1387) by graduated reduction of the corresponding nitroso-dimers; (IV; R = OEt) was also prepared by oxidation of ethyl 2-azo*iso*butyrate with hydrogen peroxide in acetic acid. By condensing the appropriate nitroso- and hydroxylamino-octanes, Aston and Ailman (*ibid.*, 1938, **60**, 1930) prepared the only known azoxy-paraffin, 2-azoxy-2 : 5-dimethylhexane (V).

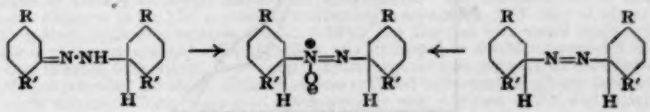


Apart from their stability to oxidising agents and lack of basic properties, Aston's compounds seem to have little in common chemically with macrozamin. Thus (V) was stable to alkali, and

was decomposed by stannous chloride and hydrochloric acid, but not by the latter alone, giving nitrogen, octenes, etc.; (IV; R = Me) was reduced by stannous chloride and hydrochloric acid giving hydrazine. This difference in behaviour is not, however, surprising, for the proposed structure (III) has two features not possessed by (IV) or (V), both of which must influence decisively the behaviour of the molecule. The first of these features is the primeverosyloxy-group of (III), which probably determines the course of the acidic hydrolysis; if, as the speed of the latter suggests, the initial attack is at the glycosidic link, then the elimination of formaldehyde and nitrogen must follow, as shown, for example, by the scheme below, in which possible intermediates are formulated:



A second feature of structure (III) is that the carbon atoms adjacent to the azoxy-group carry hydrogen atoms; until now no azoxy-compound of this kind was known. Since a similar feature in azo-compounds is known to lead to instability (isomerisation to hydrazones) it was thought desirable to show that azoxy-compounds of this kind are capable of existence; this was done as follows. Kijner (*J. Russ. Phys. Chem. Soc.*, **1899**, **31**, 894) obtained, by the action of concentrated nitric acid on menthone menthylhydrazone, a very stable compound of the composition $(menthyl)_2ON_2$ to which he tentatively assigned a structure which, if translated into modern terms, would represent 3-azoxy-*p*-menthane (VI; R = Me, R′ = Pr^i). It is shown below that this, and the analogous azoxy*cyclo*hexane (VI; R = R′ = H), now obtained by oxidising azo*cyclo*hexane with nitric acid, are true secondary azoxy-compounds; their stability is probably due to resonance in the azoxy-group.



(VI.)

The accuracy of the proposed structure (III) could now be tested; if it is correct, the synthetic azoxy-compounds (IV; R = OEt), (V), (VI; R = R′ = H), and (VI; R = Me, R′ = Pr^i) should show absorption bands in the infra-red and ultra-violet closely similar to the very characteristic bands found for macrozamin. The relevant parts of the infra-red absorption spectra of the first three of these compounds are shown in Fig. 3. Dr. Sheppard, to whom we are indebted for his advice, reports on them as follows.

" These spectra all showed a strong band in the region 1495—1505 cm.$^{-1}$ which undoubtedly arises from a vibration of the azoxy-group; no other bands were found in the region 1500—1800 cm.$^{-1}$, usually characteristic of double bonds, except in the spectrum of (IV; R = OEt), where the strong band near 1745 cm.$^{-1}$ is attributable to the ester carbonyl groups. It is probable that the band near 1500 cm.$^{-1}$ corresponds to the asymmetric stretching mode of the azoxy-group, and it is of interest that the corresponding mode of vibration of the electronically similar C–NO_2 group has a frequency of 1555—1750 cm.$^{-1}$ in the liquid state (Smith, Pan, and Nielson, *J. Chem. Phys.*, 1950, **18**, 706).

" The characteristic azoxy-frequency near 1500 cm.$^{-1}$ correlates only approximately with the absorption band of macrozamin near 1530 cm.$^{-1}$, but it must be borne in mind that the compounds so far investigated contain heavy secondary or tertiary hydrocarbon residues attached to the azoxy-group, and since the corresponding frequency of the nitro-group shifts from 1570 to 1558 cm.$^{-1}$ on passing from nitromethane to 2-nitropropane it is likely that the azoxy-group would have a considerably higher frequency if it bore a methyl group as postulated in structure (III) for macrozamin. The extent of this frequency shift could not be assessed without an investigation of azoxy-compounds bearing groups of this kind, but pending this it can be concluded that the absorption band near 1530 cm.$^{-1}$ shown by macrozamin is consistent with the presence of an azoxy-group."

The ultra-violet absorption maxima of the synthetic azoxy-compounds are listed in the Table,

and the extinction curves of the tertiary compound (V) and the secondary compound (VI; R = R′ = H) are shown in Fig. 4.

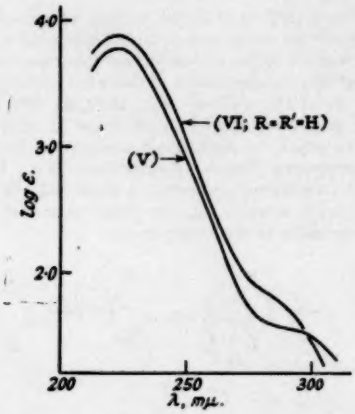
Light Absorption of Aliphatic Azoxy-compounds in Alcohol.

Compound.	$\lambda_{max.}$ (mμ.).	log ε.	Compound.	$\lambda_{max.}$ (mμ.).	log ε.
Ethyl 2-azoxy*iso*butyrate ...	223	3·74	3-Azoxy-*p*-menthane	225	3·88
2-Azoxy-2 : 5-dimethylhexane	223	3·75	Azoxy*cyclo*hexane	223·5	3·86

The curves of Fig. 4 resemble that of macrozamin closely; the maxima are near 223 mμ. and the inflexions in the region 280—285 mμ., and are thus only 5—10 mμ., removed from those of macrozamin, which is well within the range of variation found in compounds with the same

FIG. 4.

*Light absorption of azoxy*cyclo*hexane* (VI; R = R′ = H) *and 2-azoxy-2 : 5-dimethylhexane* (V) *in alcohol.*



unsaturated group but different substituents. The ultra-violet results provide strong evidence that Aston's tertiary compounds, the azoxy*cyclo*hexane derivatives, and macrozamin all possess the same chromophore.

For a conclusive proof of structure (III), a decision between the alternatives (III*a*) and (III*b*), and a proper interpretation of the behaviour of macrozamin towards alkalis and other reagents, the synthesis and examination, both chemical and spectroscopic, of a range of aliphatic azoxy-compounds more closely related to these structures than those so far prepared are necessary, and this work is in progress.

EXPERIMENTAL.

Hydrolysis of Macrozamin with Dilute Acid.—Determination of formaldehyde. Macrozamin (36·1 mg.) and N-hydrochloric acid (2 c.c.) were heated under reflux at 100° for 2 hours. The condenser was rinsed with N-sodium acetate solution (5 c.c.) and water (6 c.c.), and the solution and washings were treated with a solution of dimedone (160 mg.) in alcohol (2 c.c.), kept at 100° for 10 minutes, and then refrigerated. The derivative which separated was dried to constant weight (26·9 mg.) in a vacuum-desiccator over sulphuric acid. After recrystallisation from dilute alcohol it had m. p. 187—189°; Reeves (*J. Amer. Chem. Soc.*, 1941, **63**, 1476) gives 189—190° for the dimedone derivative of formaldehyde (Found: 0·98 mol. of formaldehyde per mol. of macrozamin).

Determination of methanol. A Kipp carbon dioxide generator was connected through a washing flask to a small hydrolysis flask from which a delivery tube led to a Zeisel apparatus of the type described by Belcher and Godbert ("Semi-micro Quantitative Organic Analysis," Longmans, Green & Co., 1945, p. 119) so modified that the gas inlet tube reached almost to the bottom of the flask containing the hydriodic acid. In the hydrolysis flask were placed macrozamin (30 mg.), acetic acid (2 c.c.), and N-hydrochloric acid (2 c.c.). The washing and the hydrolysis flask were kept at 100° during the determination, which was allowed to continue for 3 hours; the methyl iodide formed was determined iodometrically according to Belcher and Godbert's method (Found: 0·51 mol. of methanol per mol. of macrozamin). Under similar conditions methyl oxalate gave 2 × 0·66 mol. of methanol per mol. of ester.

Detection and determination of nitrogen. The macrozamin (479 mg.) was placed in a 25 c.c.-flask, which was filled almost completely with N-hydrochloric acid; the flask was then closed with a stopper having a short glass tube which was filled with air-free distilled water. The flask was submerged in a beaker of air-free water and a filled, inverted burette brought over the exit tube. The beaker was heated in a boiling-water bath till gas evolution had ceased (2 hours), the apparatus allowed to cool, and the water in the burette levelled. Corrected to N.T.P., the gas volume was 28·4 c.c., corresponding to 1·02 mols. of nitrogen per mol. of macrozamin. Standard observations on samples of gas prepared in this way, showed that it was nitrogen, uncontaminated with oxygen.

Behaviour of Macrozamin with Alkalis.—Methanolic sodium methoxide and hexa-acetyl macrozamin. A solution of the hexa-acetate (1·28 g.) in chloroform (5 c.c.) was treated with 2N-methanolic sodium methoxide (15 c.c.) and kept at room temperature for 1 hour. The white crystalline precipitate was collected, washed with a little cold methanol, dried, and heated at 100° for 1 hour with acetic acid (2 c.c.), acetic anhydride (5 c.c.), and fused sodium acetate (0·5 g.). The cooled mixture was poured into water, and, when the excess of acetic anhydride had decomposed, the solid was collected and recrystallised from alcohol, giving β-hepta-acetyl primeverose, m. p. 212—214° undepressed on admixture with authentic material.

The alkaline filtrate from which the sodium derivative of primeverose had separated contained sodium cyanide and formaldehyde; determination of the latter as its dimedone derivative showed the presence of 0·45 mol. per mol. of macrozamin.

(a) *Action of sodium hydroxide solution on macrozamin. The determination of nitrogen and formic acid.* Macrozamin (35 mg.) and N-sodium hydroxide solution (2 c.c.) were allowed to interact in an apparatus of the van Slyke type. The reaction was completed by heating the reaction tube by an external jacket through which boiling alcohol vapour was passed. When the apparatus had been cooled and the mercury levelled the gas volume was read and corrected to N.T.P.; 0·45 mol. (0·9 c.c.) was obtained per mol. of macrozamin used. The usual tests showed that the gas was nitrogen, and that it was uncontaminated by oxygen.

Alkaline reaction solutions similar to that which remained at the end of the above experiment were acidified and distilled in an apparatus of the kind used for the determination of acetyl groups (Belcher and Godbert, *loc. cit.*, p. 123); the distillates were titrated with alkali, methyl-red being used as indicator. The neutral solutions so obtained reduced ammoniacal silver nitrate solution, indicating that the volatile strong acid was formic acid, of which 0·45 mol. was obtained per mol. of macrozamin used.

(b) *Action of sodium hydroxide solution on macrozamin. The determination of volatile bases and cyanide ion.* Aliquots (5 c.c.) of a solution containing macrozamin (392 mg.) in water (25 c.c.) were mixed with sodium hydroxide solution (10 c.c.; 10%) and distilled with steam in an apparatus for the determination of acetyl groups. The distillate was collected in 0·0266N-hydrochloric acid and the excess of acid titrated with alkali; 0·18 equiv. of base was formed per mol. of macrozamin used. The alkaline solution left after the steam-distillation was acidified with dilute sulphuric acid and distilled into alcoholic silver nitrate. The precipitated silver cyanide was collected, dried, and weighed; 0·52 mol. were obtained per mol. of macrozamin used.

The volatile base obtained in the above experiment was converted into the chloroplatinate, analysis of which showed that approximately equal parts of methylamine and ammonia were present [Found: C, 3·2; H, 2·2. Calc. for $(NH_3Me)_2PtCl_6$: C, 5·1; H, 2·5. Calc. for $(NH_4)_2PtCl_6$: C, 0·0; H, 1·8%].

Action of Chloroformic Hydrogen Chloride on Hexa-acetyl Macrozamin.—A solution of the hexa-acetate (1·284 g.) in dry chloroform (20 c.c.) and dry ether (5 c.c.) was saturated with dry hydrogen chloride (30 minutes), and the solution kept at room temperature with the exclusion of moisture for 60 hours. The precipitated solid was collected, washed with ether and chloroform, and dried (160 mg.). Material prepared in this way was shown to be hydrazine dihydrochloride by the following tests: (*a*) it reduced Fehling's solution and ammoniacal silver solutions immediately in the cold with the evolution of nitrogen gas; (*b*) a sample (120 mg.) dissolved in water (0·6 c.c.) and treated with 30% sulphuric acid (0·3 c.c.) gave a sparingly soluble precipitate of hydrazine sulphate (132 mg.) which, after one recrystallisation from hot water, had m. p. 252° (decomp.) undepressed on admixture with authentic material (Found: N, 21·8. Calc. for N_2H_4,H_2SO_4: N, 21·5%); a portion of the sulphate (113·8 mg.) titrated with 0·025M-potassium iodate under Andrews's conditions required 34·8 c.c. (theoretical titre for hydrazine sulphate: 35·0 c.c.).

Titration of the crude reaction product with potassium iodate showed it was hydrazine dihydrochloride of 97% purity with respect to hydrazine. Similar titration of the chloroform filtrate revealed the presence of further small amounts of hydrazine, the total yield being 79·4%. The filtrate also contained formaldehyde, which was determined colorimetrically by the chromotropic acid method; 0·85 mol. was formed per mol. of macrozamin used; this figure includes the small amounts of formaldehyde found in the crude precipitate of hydrazine dihydrochloride.

The chloroform filtrate obtained from an experiment similar to that described above was washed with water till free from hydrochloric acid, dried, and evaporated under reduced pressure. The residue of partially acetylated sugar had strong reducing properties towards Fehling's solution, and was insoluble in sodium carbonate solution, so it was not an acetylated aldonic acid. It contained minor amounts of acetylated 1-halogeno-sugar, since some silver chloride was formed when it was boiled with alcoholic silver nitrate. When it was crystallised from alcohol, ill-defined material, m. p. 215—230° (decomp.), was obtained, which could not be purified by the use of other solvents or by chromatography. Acetylation with acetic anhydride and pyridine gave an equally ill-defined material, m. p. 185—195°, which could not be purified.

Action of Reducing Agents.—Attempted hydrogenation of macrozamin with palladium and hydrogen. A solution of macrozamin (2·334 g.) in water (50 c.c.) and alcohol (50 c.c.) containing palladous oxide (85 mg.) and a trace of palladium on charcoal was shaken in a hydrogen atmosphere. No hydrogen was absorbed either at 15° or at 50°, and when the catalyst was removed, and the solution concentrated and diluted with alcohol, the macrozamin was recovered unchanged.

Action of stannous chloride on macrozamin in cold 5N-*hydrochloric acid.* A 5·5 c.c. flask containing macrozamin (100 mg.) and A.R. stannous chloride dihydrate (572 mg.) was filled to a volume of 5·47 c.c. with 5N-hydrochloric acid, stoppered, and kept at room temperature for 7 days. A similar 5·39 c.c. flask containing stannous chloride dihydrate (560 mg.) and 5N-hydrochloric acid to a volume of 5·36 c.c. was kept for the same time as a control; titration of an aliquot iodometrically showed that no stannous chloride had been used by aerial oxidation. Iodometric titration of an aliquot from the experimental flask showed that 2·93 mols. of stannous chloride had been oxidised per mol. of macrozamin used.

An aliquot of the experimental solution (1 c.c.) was evaporated twice with water (4 c.c.) in a small Kjeldahl tube in order to remove formaldehyde, and then transferred to a Kjeldahl apparatus, made alkaline, and distilled into standard hydrochloric acid. The excess of acid was titrated with alkali, the results showing that 1·92 equivs. of volatile base were formed per mol. of macrozamin used.

The neutral solution so obtained was made up to 50 c.c. with water, and the methylamine present in an aliquot (1 c.c.) determined by Cromwell's colorimetric micro-method (*loc. cit.*). We wish to comment that Cromwell appears to carry out the oxidative deamination of methylamine at pH 5·5; at this pH we found that the reaction gives a yield of formaldehyde which is irregular and only about 50% of the theoretical, so that although a calibration curve was used, good results could not be obtained. When the oxidation was carried out at pH 7, the yield was 95% or better, and the use of a calibration curve permitted an accuracy of $\pm 3\%$. It was found that in the reduction 1·03 mols. of methylamine were formed per mol. of macrozamin used.

Formaldehyde was determined in an aliquot (2 c.c.) of the reduction solution by distillation with water and treatment of the buffered distillate with dimedone. Formaldehyde produced: 0·95 mol. per mol. of macrozamin used.

Macrozamin, platinic oxide, and hydrogen in 0·2N-*hydrochloric acid.* When macrozamin (202 mg.) was hydrogenated at room temperature in 0·2N-hydrochloric acid (25 c.c.) containing platinic oxide, 48 c.c. of hydrogen were absorbed (4·06 mols. per mol.). The filtered solution was made alkaline and distilled into hydrochloric acid. Evaporation of the latter gave a crystalline residue (74 mg.; 2 mols. of $MeNH_2$,HCl per mol. requires 71 mg.). Only traces of this material were extractable with chloroform, but it was readily and completely soluble in alcohol, so that substantial amounts of ammonium chloride or dimethylamine hydrochloride were absent. The chloroplatinate was analysed [Found: C, 5·5; H, 2·2; Pt, 43·2. Calc. for $(MeNH_3)_2PtCl_6$: C, 5·1; H, 2·6; Pt, 41·3%].

Note on Hexa-acetyl Macrozamin.—A crystalline form different from that described in Part I (*loc. cit.*) was obtained from alcohol as needles, m. p. 150—151° [Found: *M* (Rast), 631. Calc. for $C_{33}H_{34}O_{17}N_2$: *M*, 636].

3-*Azoxy*-p-*menthane.*—Made by Kijner's method (*loc. cit.*) our sample had m. p. 82°, unchanged on further recrystallisation (Found: C, 74·2; H, 11·7; N, 9·2. Calc. for $C_{20}H_{34}ON_2$: C, 74·5; H, 11·7; N, 8·7%). Kijner reports m. p. 84—84·5°.

*Azoxy*cyclo*hexane.*—Azo*cyclo*hexane (1 g.) (Harkins and Lochte, *J. Amer. Chem. Soc.*, 1924, **46**, 450) was added in small amounts during 5 minutes to vigorously stirred nitric acid (5 c.c.; *d* 1·4), stirring was continued for 1 hour and the mixture then poured into cold water (100 c.c.). Extraction with ether and evaporation of the washed and dried extract gave *azoxy*cyclo*hexane* (0·7 g.), m. p. 22—23° (Found: C, 68·5; H, 11·1; N, 13·4. $C_{12}H_{22}ON_2$ requires C, 68·6; H, 10·5; N, 13·3%).

We are indebted to Sir John Simonsen, F.R.S., of the Colonial Products Research Council and to Mr. L. J. Webb of the Commonwealth Scientific and Industrial Research Organisation for help in obtaining plant materials, to Professor J. G. Aston for a sample of 2-azoxy-2:5-dimethylhexane, to Dr. R. N. Haszeldine for spectroscopic measurements relating to Figs. 1, 2, and 4, to Dr. N. Sheppard and Mr. J. K. Brown for those of Fig. 3, to Professor A. R. Todd, F.R.S, for his interest in the work, to the Australian Council of Scientific and Industrial Research for a Senior Studentship (to N. V. R.), and to the Department of Scientific and Industrial Research for a Maintenance Grant (to B. W. L.).

UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, December 18th*, 1950.]

513. *The Kinetics of the Photolysis of Acetaldehyde. Part II.* The Rate of Production of Methyl Radicals.*

By A. S. Buchanan.

The rate of production of ethane in the high-temperature photolysis of acetaldehyde has been calculated from data on the rate of formation of methyl radicals. These results have been compared with the ethane in the reaction products as determined by analysis.

In conjunction with direct determinations of the ethane produced in the high-temperature photolysis of acetaldehyde (Danby, Buchanan, and Henderson, *J.*, 1951, 1426) determinations have been made of the rate of production of methyl radicals in the system undergoing photolysis. Such data enabled calculations of the ethane which would be produced if all the methyl radicals associated to give this substance. Comparison of the calculated values with the actual production of ethane should give a measure of the efficiency of the latter reaction.

The experimental method was that first used by Gorin (*Acta Physiochim. U.R.S.S.*, 1938, **9**, 681), and subsequently repeated and extended by Blacet and his co-workers (Blacet and Heldman, *J. Amer. Chem. Soc.*, 1942, **64**, 889; Blacet and Loeffler, *ibid.*, p. 893). Acetaldehyde is illuminated in the presence of iodine, and the methyl radicals, as they are produced, form methyl iodide which may be estimated. In addition, the formyl radicals may also react with iodine, *e.g.*, $CHO + I_2 = HI + CO + I$. Some evidence for the latter reaction was obtained by Gorin but the amounts of hydrogen iodide produced were very small, being only 1/10th to 1/100th of the methyl iodide production. Blacet was unable to detect hydrogen iodide in measurable amount and suggested that it probably reacts with acetaldehyde to give polymers.

Experimental.

The experimental arrangements were the same as those described by Danby, Buchanan, and Henderson (*loc. cit.*). The required pressure of iodine was vaporized into the reaction vessel, and the acetaldehyde then admitted. The vessel was isolated by a magnetically operated ground-glass valve. After illumination, the reaction products were pumped slowly (with a Töpler pump) over a large area of mercury on the walls of the tube, to remove excess of iodine. The products were then compressed in the absorbing section of the apparatus, which consisted of a U-tube containing potassium hydroxide pellets and a tube containing a measured volume of sodium ethoxide solution. The former was intended to remove hydrogen iodide, but the amount detected was always negligible and, since the potassium hydroxide sometimes absorbed appreciable amounts of methyl iodide, the use of this tube was dispensed with in most cases. After being sealed, the sodium ethoxide tube was heated at 130° for several hours, and the sodium iodide produced was determined.

Results.

In the first instance a survey of the method was made, *i.e.*, determination of the methyl-radical production with variation in acetaldehyde pressure, time of illumination, pressure of iodine, and temperature.

Fig. 1(*a*) shows the amount of methyl iodide produced as the pressure of aldehyde increases at 60 minutes' illumination and approximately 5 mm. of iodine. Above about 3×10^{-4} mole of acetaldehyde per 105 c.c., the production of methyl iodide ceases to increase with pressure, remaining constant thereafter. To ensure that this effect was not due to lack of iodine (since the methyl iodide might decompose if excess of iodine was not present) a similar curve but with double the iodine pressure, was prepared [Fig. 1(*b*)]. There are apparently no significant differences in the curves; the fact that the plateau occurs at a somewhat higher value in the latter is almost certainly to be ascribed to experimental error. Halving the exposure time to 30 minutes gave a curve with much the same characteristics [Fig. 1(*c*)]. The slope of the initial proportional region is just half that for the 60-minute curve and the plateau value is slightly over half. The beginning of the plateau in each case occurred at an aldehyde concentration of about 3—4 $\times 10^{-4}$ mole per 105 c.c. The evidence thus suggested that the appearance of the plateau was due to complete utilisation of the light entering the cell, and this was confirmed by arranging a reflector behind the lamp in order to increase the incident intensity, whereupon an increase in methyl iodide production of 30% was observed.

The influence of time of exposure on methyl-radical production was investigated at 120° and 200° (Fig. 2). A concentration of aldehyde sufficiently high to secure full light utilisation was used throughout. It can be seen that, initially, the formation of methyl iodide is proportional to the time of exposure, but after about 20—30 minutes it begins to fall off. This is probably to be attributed to the effects of secondary reactions of unknown nature. However, it is clear that only results obtained with short

* Part I, *J.*, 1951, 1426.

times of exposure are unambiguous (*i.e.*, up to about 10 minutes). The initial rate of production of methyl radicals is about $0{\cdot}53 \times 10^{-6}$ and $0{\cdot}55 \times 10^{-6}$ mole per 105 c.c. per minute at 120° and 200°, respectively. This result appears to be in agreement with the generally accepted notion that radical production by photolysis should be temperature independent. At 300° an appreciable "dark" reaction occurred, apparently leading to the formation of methyl iodide; experiments at this temperature therefore were discontinued.

FIG. 1.

Production of CH_3I *with varying* $CH_3{\cdot}CHO$ *pressure:* (*a*) 5—6 *mm. of iodine;* 60 *mins.' exposure;* 120°. (*b*) 10—11 *mm. of iodine;* 60 *mins.' exposure;* 120°. (*c*) 5—6 *mm. of iodine;* 30 *mins.' exposure;* 120°.

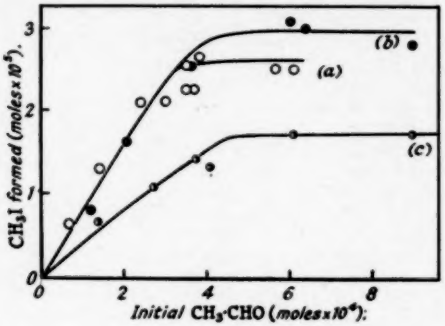
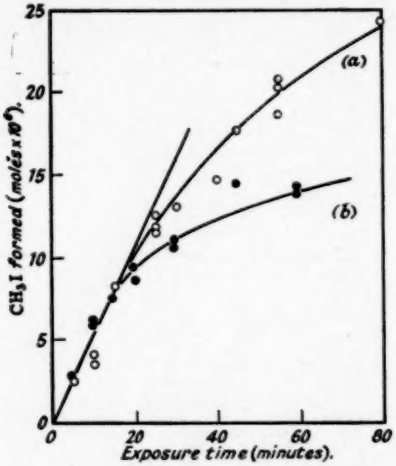


FIG. 2.

Production of CH_3I *with varying exposure time:* (*a*) *at* 120°; (*b*) *at* 200°.



To permit of a calculation of ethane production in the region of chain reaction, it is necessary to know the rate of methyl-radical formation over a range of pressures, since the aldehyde pressure rapidly diminishes during the course of an experiment. A series of pressures was therefore selected, and the initial rates of methyl iodide formation determined (Fig. 3). This curve was determined at 200°, and for use at other temperatures the reasonable assumption was made that radical production was independent of temperature. To apply it, aldehyde pressures are first reduced to values at 200°.

The method of calculation of ethane production was as follows. The period of the reaction was divided into a number of short time intervals (such that the change of aldehyde pressure was not more than about 2%), the rate of formation of methyl radicals for the mean pressure of the interval read off

from Fig. 3, and the total of methyl radicals produced during the interval obtained in this way. The sum of all these gave the total over the period of the reaction, and half this quantity represented the expected amount of ethane. For short time divisions, the errors of this method cancel, since the rate of formation of methyl radicals is greater at the beginning and less at the end of each interval, than the value for the mean pressure.

FIG. 3.

Initial rates of production of CH_3I *with varying* $CH_3{\cdot}CHO$ *pressure at* 200°.

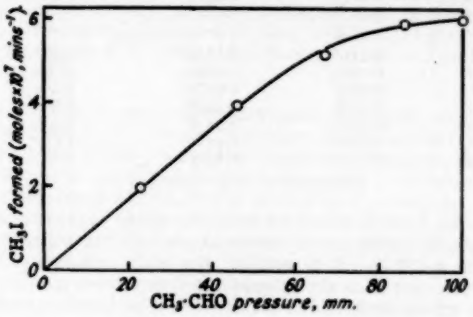


FIG. 4.

Variation in the ratio C_2H_6 *to* CH_4 *with change in* $CH_3{\cdot}CHO$ *pressure. Open circles: from mass-spectrometer analysis. Full circles: calculated from rate of formation of methyl radicals.*

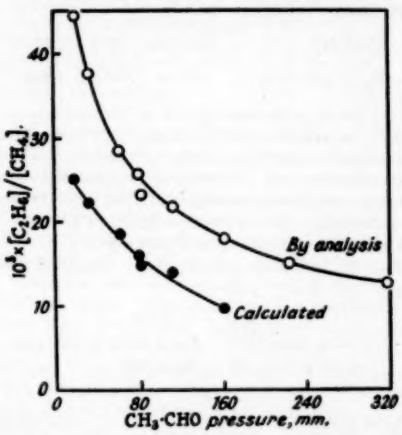
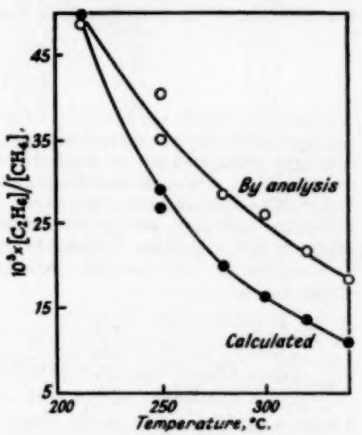


FIG. 5.

Variation in the ratio C_2H_6 *to* CH_4 *with change in temperature. Open circles: from mass-spectrometer analysis. Full circles: calculated from rate of formation of methyl radicals.*



DISCUSSION.

The calculated ratios of ethane to methane in the products are given in the Table, together with the results obtained by the mass-spectrometer analysis. Consideration of the ratio of ethane to methane (col. 3 and Fig. 4) indicates that, as the pressure of aldehyde is increased (at one temperature), the proportion of ethane decreases since the chain length is increased and more methane molecules are produced per methyl radical. The decrease in the proportion of ethane with rising temperature may be explained in the same way, since the chain length is considerably increased on going from 212° to 340°. Further, it will be noted that the calculated ratios of ethane to methane (col. 4 and Figs. 4 and 5) vary in much the same way, suggesting that the formation of ethane takes place by the association of two methyl radicals.

Press. of CH_3·CHO, mm.	Temp.	Ratio of C_2H_6 to CH_4 in product. Found.	Calc.	Ratio of actual to calculated C_2H_6.	Ratio of H_2 to C_2H_6 in product.
19·4	300°	0·0445	0·0247	1·80	1·20
30·8	300	0·0376	0·0222	1·70	—
61·5	300	0·0286	0·0187	1·53	—
80·0	300	0·0262	0·0161	1·62	—
112·0	300	0·0218	0·0143	1·53	—
161·2	300	0·0182	0·0094	1·94	1·17
224·0	300	0·0150	—	—	—
317·3	300	0·0129	—	—	1·16
81·1	212	0·0487	0·0500	0·98	—
81·3	250	0·0408	0·0298	1·36	—
82·5	250	0·0345	0·0273	1·26	1·11
83·0	280	0·0290	0·0198	1·47	—
82·0	300	0·0227	0·0146	1·55	—
80·0	320	0·0218	0·0136	1·60	—
80·0	340	0·0188	0·0106	1·77	—

Illumination time : 8 minutes.

It is evident, however, that in almost all cases the actual production of ethane is in excess of that calculated from the iodine experiments—the ratio of these quantities varies from 0·98 to 1·94 (col. 5 and Figs. 4 and 5). At about 200°, the actual and calculated amounts of ethane are approximately the same, but as the temperature rises there is a progressive increase in the ratio until at 340° the ethane formed is about 80% in excess of that calculated. This suggests that a proportion (increasing with temperature) of the formyl radicals react to give methyl radicals by a process such as

$$CHO = H + CO \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)\ *$$

$$H + CH_3{\cdot}CHO = H_2 + CH_3 + CO \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (9)\ *$$

Each formyl radical yields a molecule of hydrogen by this process. It seems probable that the remainder of the formyl radicals undergo an alternative reaction :

$$CHO = H + CO \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

$$H + H + (M) = H_2 + (M) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (10)$$

Two formyl radicals are necessary to produce a molecule of hydrogen by the latter mechanism. Analysis for hydrogen showed that, within the limits of accuracy of the analysis, ethane and hydrogen were produced in practically equivalent amounts under a variety of conditions (last col. in table). The simultaneous occurrence of reactions (9) and (10) is quite compatible with this result, since reaction (9), which yields the required extra methyl radicals, also yields additional hydrogen, and it will be found that hydrogen equivalent to ethane is produced whatever the proportion of formyl radicals reacting by the two alternative processes.

Summing up, the reactions occurring in the high-temperature photolysis of acetaldehyde appear to be :

$$CH_3{\cdot}CHO + h\nu = CH_4 + CO \qquad HCO = H + CO$$

$$CH_3{\cdot}CHO + h\nu = CH_3 + CHO \qquad H + CH_3{\cdot}CHO = H_2 + CH_3 + CO$$

$$CH_3 + CH_3{\cdot}CHO = CH_4 + CH_3 + CO \qquad H + H(+ M) = H_2(+ M)$$

$$2CH_3 = C_2H_6$$

Although these reactions are in accord with the data presented in this paper, it is considered desirable that the investigations be continued at temperatures below 200°, and the results may necessitate modification of the above views.

The author records his appreciation of many helpful discussions with Sir Cyril Hinshelwood, F.R.S., throughout the course of this work, which was carried out whilst on leave from the Chemistry Department, University of Melbourne.

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* Equations are numbered to correspond with those in Part I (*loc. cit.*).

514. *The Dipole Moments of Some Derivatives of Diphenylmethanol and Related Compounds.*

By D. Cleverdon and J. W. Smith.

From measurements of the dielectric constants, refractive indices, and specific volumes of solutions in benzene at 25° the following apparent dipole moments have been deduced; diphenylmethanol 1·60, *p*-methoxy- 2·03, *op*-dimethoxy- 2·69, *mp*-dimethoxy- 2·11, and *p*-phenoxy-diphenylmethanol 1·91, *op'*-ditolylmethanol 1·73, *op*-dimethoxybenzophenone 4·16, and diphenylchloromethane 1·85 D.

The measurements recorded here were commenced some years ago, when it was considered possible that the different behaviours of the esters of diphenylmethanol derivatives on hydrolysis (alkyl–oxygen and acyl–oxygen fission) might be reflected in the dipole moments of the alcohols. No very distinct displacement of the apparent moments in a direction parallel with the hydrolytic behaviour of the esters was found, but as there are no recorded moments of any of the compounds studied except diphenylchloromethane, the measurements have some interest in other connexions. The results are summarised in Table I, where α and β are the limiting values of $\Delta\varepsilon/w_2$ and $\Delta v/w_2$, respectively, at zero concentration, and the other symbols have their usual significance. The value obtained for diphenylchloromethane is in accord with that reported by Weissberger and Sangewald (*Z. physikal. Chem.*, 1933, *B*, **20**, 150), although they found $P_{2\infty}$ and $[R]$ to be 138 and 64·0 c.c., respectively.

Table I.

	α.	β.	$P_{2\infty}$, c.c.	$[R]$, c.c.	μ (D). $P_{E+A} = [R]$.	μ (D). $P_{E+A} = 1{\cdot}05\,[R]$
Diphenylmethanol	1·80	−0·2498	111·5	56·7	1·64	1·60
p-Methoxydiphenylmethanol	2·38	−0·2774	151·2	63·7	2·07	2·03
op-Dimethoxydiphenylmethanol	3·51	−0·3031	222·5	71·5	2·72	2·69
mp-Dimethoxydiphenylmethanol	2·28	−0·3050	165·9	(71·5)	2·15	2·11
p-Phenoxydiphenylmethanol	1·77	−0·2955	161·3	83·1	1·96	1·91
op'-Ditolylmethanol	1·78	−0·2108	130·0	(67·7)	1·78	1·73
op-Dimethoxybenzophenone	8·10	−0·3141	429·0	71·8	4·18	4·16
Diphenylchloromethane	2·17	−0·2927	134·2	61·3	1·89	1·85

The molecular polarisations of the alcohols, particularly of diphenyl- and *p*-methoxydiphenyl-methanol, show a tendency to decrease at the lowest concentrations, and the plot of $\Delta\varepsilon/w_2$ against w_2 is not linear. Extrapolation of the results by any method is therefore subject to inaccuracies. This behaviour has been found to be common with alcohols, and is attributable to association which increases with increasing concentration. It may be expected, however, that extrapolation of the results for low concentrations should yield approximate values for the monomer.

Owing to this circumstance, and to the fact that some of the compounds studied probably have high atomic polarisations and a considerable solvent effect, any inferences which might be drawn from the rather complex vectorial analyses of these moments would be hazardous. Qualitatively, however, it is of interest to note that the dipole moment of diphenylmethanol is less than that reported for benzyl alcohol (1·69) * and much lower than for triphenylmethanol (2·11), whereas the value for diphenylchloromethane lies intermediately between those for benzyl chloride (1·82) and triphenylchloromethane (1·925) and is almost equal to that of methyl chloride (1·86). The remaining moments determined are consistent with other data; *e.g.*, the value of *p*-phenoxydiphenylmethanol is slightly lower than that of the *p*-methoxy-analogue, as is to be expected from the lower moment of diphenyl ether (1·1) as compared with anisole (1·23).

Experimental.

Materials.—Diphenylmethanol was prepared by reduction of vacuum-redistilled commercial benzophenone with zinc dust and sodium hydroxide in ethyl alcohol (*Org. Synth.*, 1941, *Coll. Vol.* **1**, 90). The crude product, extracted with light petroleum (b. p. 60—80°) in a Soxhlet apparatus and recrystallised twice from light petroleum, had m. p. 68·2°.

* All dipole moments quoted are taken from the Massachusetts Institute of Technology Tables (1948) and are values (in Debye units) for benzene solution.

TABLE II.

Polarisation data for benzene solutions.

$100w_2$.	ε_{12}.	v_{12}.	β_{12}. c.c.	P_2, c.c.	n.	r_{12}, c.c.	$[R]$, c.c.
0·0000	**2·2725**	**1·14458**	**0·34090**	—	1·4980 *	0·33550	—
					1·5021 †	0·33785	
			Diphenylmethanol.				
1·6514	2·3022	1·1405	0·34521	110·9	—	—	—
1·8538	2·3072	1·1399	0·34595	112·9	—	—	—
3·5307	2·3390	1·1358	0·35050	112·9	—	—	—
3·8758	2·3451	1·1351	0·35139	112·7	—	—	—
4·9149	2·3669	1·1324	0·35445	113·6	—	—	—
1·4848	2·2995	1·14089	0·34485	111·5	1·4991 *	0·33504	56·2
3·1834	2·3322	1·13664	0·34953	112·8	1·5005 *	0·33459	56·5
4·6670	2·3615	1·13291	0·35365	113·1	1·5019 *	0·33428	57·0
6·2586	2·3934	1·12897	0·35805	113·3	1·5031 *	0·33378	56·7
			p-Methoxydiphenylmethanol.				
1·4904	2·3084	1·14045	0·34634	151·3	1·4991 *	0·33491	63·5
2·7313	2·3391	1·13697	0·35088	151·4	1·5001 *	0·33446	63·7
4·0296	2·3712	1·13349	0·35556	151·0	1·5010 *	0·33394	63·6
6·6676	2·4410	1·12607	0·36538	151·7	1·5032 *	0·33298	63·8
			op-Dimethoxydiphenylmethanol.				
2·2835	2·3527	1·13757	0·35353	218·5	1·5040 †	0·33686	71·9
2·5805	2·3638	1·13676	0·35527	219·4	1·5042 †	0·33673	71·9
6·5392	2·5049	1·12483	0·37576	213·6	1·5071 †	0·33482	71·2
			mp-Dimethoxydiphenylmethanol.				
0·8264	2·2912	1·14206	0·34370	166·0	—	—	—
1·3713	2·3038	1·14040	0·34547	164·7	—	—	—
2·7888	2·3361	1·13607	0·35006	163·5	—	—	—
3·7375	2·3572	1·13317	0·35296	162·1	—	—	—
			p-Phenoxydiphenylmethanol.				
2·338	2·3149	1·13766	0·34668	161·9	—	—	—
2·804	1·136	1·13629	—	—	1·5008 *	0·33466	84·1
5·507	2·3758	1·12831	0·35476	163·1	1·5030 *	0·33354	82·6
8·294	2·4333	1·11995	0·36208	164·2	—	—	—
11·017	—	1·11208	—	—	1·5087 *	0·33178	83·1
			op'-Ditolylmethanol.				
0·528	2·2820	1·14343	0·34233	130·0	—	—	—
0·915	2·2879	1·14277	0·34324	126·7	—	—	—
1·870	2·3083	1·14069	0·34639	134·7	—	—	—
5·089	2·3664	1·13368	0·35477	130·3	—	—	—
			op-Dimethoxybenzophenone.				
1·8729	2·4250	1·13855	0·36665	415·6	1·5040 †	0·33715	73·8
2·8252	2·5043	1·13564	0·37927	411·6	1·5047 †	0·33648	70·1
5·4466	2·7149	1·12740	0·41007	390·3	1·5072 †	0·33565	72·1
8·8817	3·0128	1·11662	0·44863	376·4	1·5104 †	0·33420	71·9
			Diphenylchloromethane.				
5·1015	2·3850	1·1298	0·35685	132·5	1·5073 †	0·33638	62·6
6·1520	2·4125	1·1266	0·36064	134·2	1·5081 †	0·33586	62·0
11·6380	2·5474	1·1104	0·37785	133·5	1·5120 †	0·33321	60·4

* Sodium-D line. † Mercury green line.

p-Methoxydiphenylmethanol was prepared by interaction of phenylmagnesium bromide with anisaldehyde, the addition product being decomposed with ammonium chloride and ice. The dried ethereal extract was concentrated, and the carbinol precipitated with light petroleum. It was recrystallised from light petroleum.

The remainder of the solutes were kindly presented by Dr. J. Kenyon and his co-workers, and were recrystallised before use.

Thiophen-free benzene was fractionally crystallised, dried over phosphoric oxide, and redistilled immediately before use.

Apparatus and Methods.—For the second series of solutions of diphenylmethanol and for its *p*-methoxy-derivative the dielectric constants were measured with a heterodyne-beat apparatus, but for the remainder, which were studied during the war when other equipment was not obtainable, the apparatus used was similar to that described by Le Fèvre and Rayner (*J.*, 1938, 1921), but with the screened grid valve replaced by a pentode which increased the sensitivity. Specific volumes were determined with a pyknometer, and the refractive indices with a Pulfrich refractometer. All measurements were made at 25·0°.

Details of the measurements are shown in Table II, where the symbols used have their usual significance. The P_{2_∞} values for the solutes shown in Table I were calculated by the method described previously (*Trans. Faraday Soc.*, 1949, **45**, 109) and are consistent with the P_2 values for finite concentrations. For *op'*-ditolylmethanol the molecular refraction was calculated from the bond refractions (Vogel *et al.*, *Chem. and Ind.*, 1950, 358), whilst for *mp*-dimethoxydiphenylmethanol the value was taken as equal to that for the *op*-isomer.

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515. *Substitution Reactions of Pyrimidine and its 2- and 4-Phenyl Derivatives.*

By B. LYTHGOE and L. S. RAYNER.

Factors which may influence the course of catalytic dehalogenation of chloropyrimidines are considered and conditions for the preparation of pyrimidine itself on a half-molar scale from its 2 : 5-dichloro-compound are defined. Pyrimidine resisted attempted nitration; its 2-phenyl derivative underwent nitration *meta* in the benzene nucleus; its 4-phenyl derivative probably behaves similarly. The behaviour of pyrimidine with free *p*-nitrophenyl radical in the Gomberg–Hey reaction has been studied and compared with that of pyridine.

THE present work was prompted by a wish to find out how far the analogy between pyrimidine and pyridine derivatives (compare Lythgoe, *Quart. Reviews*, 1949, **3**, 194) is valid for the parent compound pyrimidine and its alkyl and aryl derivatives. The behaviour of pyridine towards electrophilic, nucleophilic, and free-radical reagents is well known and highly characteristic; little more is known about pyrimidine in this connection than that the nucleophilic substitution of its 4-methyl derivative by sodamide parallels that of pyridine, giving a 2-amino- and a 2 : 6-diamino-compound (Ochiai and Karii, *J. Pharm. Soc. Japan*, 1939, **59**, 18). It therefore seemed of interest to study the effect of a free-radical substituting agent on pyrimidine and also to determine whether the nucleus is as resistant to electrophilic substitution as might be expected theoretically.

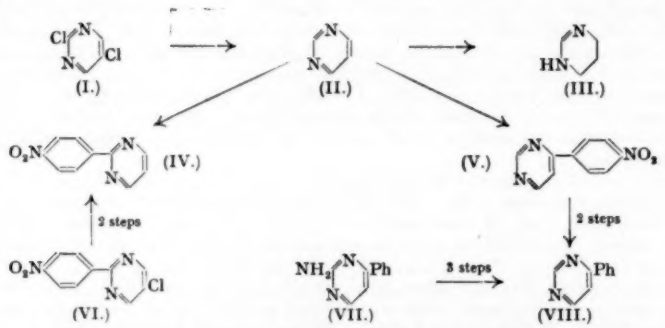
Pyrimidine was first prepared by reducing its 2 : 4 : 6-trichloro-derivative with zinc dust and water (Gabriel and Colman, *Ber.*, 1900, **33**, 3666), and more recently Cavalieri and Bendich (*J. Amer. Chem. Soc.*, 1950, **72**, 258) obtained it by desulphurising 2 : 6-dimercaptopyrimidine with Raney nickel. These and the other known methods give poor yields, and would probably not be suitable for its preparation in quantity. At the outset of this work a direct synthesis from formamidine and β-ethoxyacraldehyde acetal was contemplated, but in model experiments this acetal could not be condensed with benzamidine, and soon afterwards Price and Zomlefer (*J. Org. Chem.*, 1949, **14**, 210) reported that it did not condense with acetamidine. Attention was therefore given to the catalytic reductive dehalogenation of chloropyrimidines.

This method has been used to prepare many aminopyrimidine derivatives but is less successful in its application to true pyrimidines, *i.e.*, those carrying only alkyl or aryl substituents, where strict neutrality of the medium may be necessary to avoid both halogen replacement by solvent action and nuclear hydrogenation. Dihydroiminopyrimidines (2-, 4-, or 6-aminopyrimidines) are only difficultly hydrogenated in the nucleus; the reduction of 4-amino-6-chloro-2-methylpyrimidine stops at the stage of 4-amino-2-methylpyrimidine even when hydrochloric acid is present (Földi, Fodor, Demjen, Szekeres, and Halmos, *Ber.*, 1942, **75**, 755); on the other hand the halogen atoms in compounds such as 2-amino-4-chloropyrimidine are relatively unreactive, so that their reduction can usually be carried out

safely in the presence of alkali (cf. Roblin, Williams, Winnek, and English, *J. Amer. Chem. Soc.*, 1940, **62**, 2002). Nuclear hydrogenation of true pyrimidines is catalysed by acids, as now shown for pyrimidine itself, so that for success in dehalogenation of their chloro-derivatives the presence of a base is desirable to neutralise the hydrogen chloride set free in the reaction. Thus 5-chloro-2-phenylpyrimidine absorbed 3 molar proportions of hydrogen in the presence of palladised barium sulphate, giving 1 : 4 : 5 : 6-tetrahydro-2-phenylpyrimidine, identical with the product obtained by Aspinall (*J. Amer. Chem. Soc.*, 1940, **62**, 2160), who cyclised monobenzoyltrimethylenediamine by heating it with calcium oxide. Davies and Piggott (*J.*, 1945, **348**) observed a similar reduction of 4-chloro-5-phenylpyrimidine to a tetrahydro-5-phenylpyrimidine in the presence of palladised calcium carbonate; here the nuclear reduction appears to be promoted even by the weak carbonic acid, and a similar reduction encountered in the present work is recorded below. In view of these results it is rather surprising that Miyaki and Kataoka (*J. Pharm. Soc. Japan*, 1940, **60**, 367) were able to prepare 4-phenylpyrimidine and 4-methylpyrimidine by dehalogenating their 2 : 6-dichloro-compounds in the presence of palladised calcium carbonate; what yields were obtained is not known to us, as we were able to consult their paper only in abstract.

The suppression of nuclear reduction in the presence of aqueous sodium hydroxide was shown by the successful reduction of 5-chloro-2-phenylpyrimidine to 2-phenylpyrimidine and of 4-chloro-5-phenylpyrimidine to 5-phenylpyrimidine. Where the compound to be dehalogenated contains a halogen atom more reactive than those in the above examples, and especially when di- and tri-chloro-compounds are concerned, the presence of even quite dilute sodium hydroxide solution causes solvolytic replacement of chlorine, so that poor yields are obtained. The disparity between the hydrogen uptake and the amount of chloride ion liberated in earlier unsuccessful attempts in this laboratory to prepare pyrimidine by catalytic dehalogenation of its 2 : 4 : 6-trichloro- and its 2 : 6-dichloro-derivatives is one example of the incidence of this replacement reaction; another is mentioned below.

The above considerations dictated the preference for 2 : 5-dichloropyrimidine (I) rather than its 2 : 6-isomer as starting material for the preparation of pyrimidine; the chlorine atom at position 5 in (I) is quite stable and that at 2 not unduly reactive towards water and alcohols. The preparation of (I), although tedious, can be carried out in quantity; guanidine and tetrachloropropene condense to give 2-amino-5-chloropyrimidine (Roblin, Winnek, and English, *J. Amer. Chem. Soc.*, 1942, **64**, 567), the amino-group of which can then be replaced successively by hydroxyl and chlorine (English, Clark, Shepherd, Marson, Krapcho, and Roblin, *ibid.*, 1946, **68**, 1039).



Reduction of (I) with hydrogen and palladised barium sulphate in dilute sodium hydroxide solution was unsatisfactory, because some 5-chloro-2-hydroxypyrimidine was formed, and similarly the use of methanol containing suspended barium oxide gave 5-chloro-2-methoxypyridimidine. When water, alone or containing suspended calcium carbonate, was used as solvent 4 molar proportions of hydrogen were absorbed without any definite break in the hydrogenation curve, giving a tetrahydropyrimidine, characterised as its crystalline oxalate. It is formulated as 1 : 4 : 5 : 6-tetrahydropyrimidine (III) by analogy with the 1 : 4 : 5 : 6-tetrahydro-2-phenylpyrimidine mentioned above. Satisfactory results were obtained when (I) was hydrogenated in water containing suspended magnesium oxide. The rate of hydrogen uptake decreased after 2 molar proportions had been absorbed, and by stopping the reaction then

pyrimidine (II) was obtained as the main product, contaminated only by small amounts of 5-chloropyrimidine and of tetrahydropyrimidine. The latter was eliminated by precipitating the pyrimidine as its mercuric chloride complex in weakly acidic solution; the tetrahydro-compound forms a complex which is soluble below pH *ca.* 5. The pyrimidine was then regenerated and obtained pure in about 45% yield by fractional distillation. Pyrimidine should not be dried over potassium hydroxide, which slowly decomposes it; when heated with 30% potassium hydroxide solution it gave 1·8 mols. of volatile base (mainly ammonia).

As a way of determining which nuclear positions of pyrimidine are most susceptible to attack by free-radical reagents it was subjected to a Gomberg–Hey reaction, the mechanism of which has been established by Hey and his colleagues (for references, see " Organic Reactions," Vol. II, Chap. 6). To secure good yields and readily crystallising products diazotised *p*-nitroaniline was used as the second component, and because pyrimidine is neutral the reaction was carried out in the presence of sodium acetate (Elks, Haworth, and Hey, *J.*, 1940, 1285). Chromatography of the product on aluminium oxide gave two pure compounds, namely, 2-*p*-nitrophenylpyrimidine (IV) (yield, 10%) and 4-*p*-nitrophenylpyrimidine (V) (yield, 14%). The identification of (IV) was effected by an independent synthesis as follows. Chloromalondialdehyde and *p*-nitrobenzamidine condensed to give 5-chloro-2-*p*-nitrophenylpyrimidine (VI) which was reduced to 2-*p*-aminophenylpyrimidine; this was diazotised and converted into the corresponding nitro-compound (IV) by the method of Hodgson, Mahadevan, and Ward (*J.*, 1947, 1392).

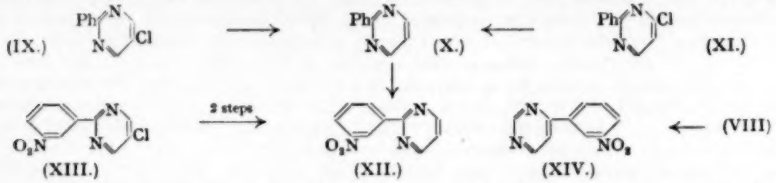
2-Amino-4-phenylpyrimidine (VII) can be made by condensing guanidine and benzoylacetaldehyde (Sprague, Kissinger, and Lincoln, *J. Amer. Chem. Soc.*, 1941, **63**, 3028) and, as shown below, can be used as starting material for a synthesis of 4-phenylpyrimidine. A similar synthesis of (VI) through 2-amino-4-*p*-nitrophenylpyrimidine from guanidine and *p*-nitrobenzoylacetaldehyde was projected, and to this end the latter compound was prepared from ethyl formate and *p*-nitroacetophenone, but it could not be caused to condense with guanidine. The structure of (VI) was therefore established by reducing it to 4-*p*-aminophenylpyrimidine, which when diazotised and treated with hypophosphorous acid gave 4-phenylpyrimidine (VIII). The latter has been described by Miyaki and Kataoka (*loc. cit.*), but for the present work it was obtained by exchanging the amino-group of (VII) successively by hydroxyl, chlorine, and hydrogen, which gave a product identical with that obtained from (V). The same compound was also obtained in low yield from the products of decomposition of *N*-nitrosoacetanilide in pyrimidine solution (cf. Haworth, Heilbron, and Hey, *J.*, 1940, 349, 373).

The reaction between pyridine and diazotised *p*-nitroaniline (Haworth, Heilbron, and Hey, *loc. cit.*) gave a product from which, by fractional crystallisation, *p*-nitrophenyl derivatives of pyridine were isolated in approximately the following yields: 2-isomer, 14·5%; 3-isomer, 5%; 4-isomer, 2·5%. The nuclear position adjacent to the nitrogen atom is here the most susceptible to attack, and on this basis the results obtained with pyrimidine are readily understood. Position 2 in the pyrimidine nucleus is adjacent to, and activated by, both nitrogen atoms, so that it is the most reactive position. Positions 4 and 6, each adjacent to one of the nitrogen atoms, are next in order of reactivity, and since substitution at either position gives the same product (V), this becomes the major product of the reaction. It would probably be unwise to attach too much significance to the failure to isolate any 5-*p*-nitrophenylpyrimidine, because of the small scale of the experiment, but it is clear that it cannot have been formed at all extensively.

Towards electrophilic substitution the pyrimidine nucleus should, theoretically, be even more resistant than that of pyridine, and it is probably significant that such substitutions have been carried out only on those pyrimidine derivatives which contain at least one activating hydroxyl, amino-, or similar group. The nitration of pyrimidine was attempted under conditions similar to those which have been used successfully for pyridine, but no nitro-compound could be isolated, so in order to secure more positive confirmation of the inertness of the nucleus the nitration of 2- and 4-phenylpyrimidines was investigated.

A compound described as 2-phenylpyrimidine was obtained by Cherbuliez and Stavritch (*Helv. Chim. Acta*, 1922, **5**, 267) who cyclised benzylideneasparagine with hypobromite to give 5-bromo-6-hydroxy-2-phenylpyrimidine and then replaced the substituents at positions 5 and 6 by hydrogen. As this method seemed inconvenient, alternatives were sought. Reductive deamination of 5-amino-2-phenylpyrimidine, obtained by reducing 5-nitro-2-phenylpyrimidine, was not found possible, since the amino-compound could not be diazotised to give a compound capable, for example, of coupling with β-naphthol. Instead, a satisfactory method was found in the catalytic reduction of 5-chloro-2-phenylpyrimidine (IX) obtained by condensing

benzamidine and chloromalondialdehyde. The 2-phenylpyrimidine (X) obtained in this way was different from that described by the Swiss workers, but its authenticity was shown by two further methods of synthesis, each of which gave the same product. In the first, the 1 : 4 : 5 : 6-tetrahydro-2-phenylpyrimidine mentioned earlier was dehydrogenated in good yield by heating



it with platinised charcoal in a current of carbon dioxide; in the second, 4-chloro-2-phenylpyrimidine was catalytically dehalogenated. Cherbuliez and Stavritch's compound cannot have the structure assigned to it, and the intermediates from which it was obtained must be regarded with some reserve.

Only one pure compound could be isolated from the product of nitration of (X); it was identified as 2-*m*-nitrophenylpyrimidine (XII) by an independent synthesis. Chloromalondialdehyde and *m*-nitrobenzamidine condensed to give 5-chloro-2-*m*-nitrophenylpyrimidine (XIII) which was reduced to 2-*m*-aminophenylpyrimidine and the amino-group was replaced by a nitro-group. The formation of (XII) from (X) made it likely that the unidentified nitro-compound which Ochiai *et al.* (*J. Pharm. Soc. Japan*, 1939, **59**, 462) obtained by nitrating 4 : 6-dimethyl-2-phenylpyrimidine would prove to be 4 : 6-dimethyl-2-*m*-nitrophenylpyrimidine, and not 4 : 6-dimethyl-5-nitro-2-phenylpyrimidine, under which name it is, for reasons which are not clear, indexed in *Chemical Abstracts* (1940, **34**, 102). This view was shown to be correct by a synthesis of the same compound from *m*-nitrobenzamidine and acetylacetone. Again only one pure compound could be isolated (and that in only moderate yield) from nitration of 4-phenylpyrimidine. Although its structure has not been proved completely it is almost certainly 4-*m*-nitrophenylpyrimidine (XIV); it differs from 4-*p*-nitrophenylpyrimidine and, in view of the results presented above, the possibility that the nitro-group entered the pyrimidine nucleus seems remote.

It is of interest to compare these results with those obtained when 2- and 4-phenylpyridines are nitrated (Forsyth and Pyman, *J.*, 1926, 2912; cf. Schofield, *Quart. Reviews*, 1950, **4**, 400). Substitution then occurs in the benzene, not the pyridine, nucleus and, although *op*-substitution predominates, considerable amounts (*ca.* 30%) of *m*-nitrophenyl derivatives are formed. The phenylpyrimidines also undergo substitution in the benzene nucleus preferentially, but apparently the deactivating effects of both nitrogen atoms are together strong enough to suppress *op*-substitution almost completely, and the *m*-nitrophenyl derivatives are the major products, just as happens with 2 : 4 : 6-triphenyl-1 : 3 : 5-triazine, which is nitrated to a tri-*m*-nitrophenyltriazine (Claus, *J. pr. Chem.*, 1895, [ii], **51**, 399).

Experimental.

5-Chloropyrimidines.—*2 : 5-Dichloropyrimidine.* Condensation of tetrachloropropene and guanidine in 20% fuming sulphuric acid (Roblin *et al.*, *loc. cit.*) gave 2-amino-5-chloropyrimidine (yield, 83%) which was converted into the dichloro-compound (yield, 50%) by a slight modification of the two-step method of English *et al.* (*loc. cit.*).

5-Chloro-2-phenylpyrimidine. After many trials, the following method was found to give the best results. Chloromalondialdehyde (5 g.; Cornforth *et al.*, *J.*, 1949, 1550) and benzamidine hydrochloride dihydrate (9·2 g.) were dissolved in methanol (50 c.c.) to which 6·2% aqueous sodium hydroxide was added (26·5 c.c.). The precipitate was collected after 16 hours; the filtrate was adjusted to pH 3·9 by addition of concentrated hydrochloric acid, kept for 15 minutes, and then adjusted to pH 10 by the addition of 10% aqueous sodium hydroxide. A further precipitate formed slowly; it was collected after 20 hours, and the filtrate retreated as before. This process was repeated four times. The combined precipitates (3·54 g.) crystallised from dilute methanol giving the pure chloro-compound, m. p. 96° (Found: C, 63·2; H, 4·1; N, 14·7. Calc. for $C_{10}H_7N_2Cl$: C, 63·0; H, 3·7; N, 14·7%).

5-Chloro-2-m-nitrophenylpyrimidine. Chloromalondialdehyde (2·44 g.) and *m*-nitrobenzamidine hydrochloride (4·61 g.) were condensed in the manner described above, and the crude product (yield, 40%) sublimed in a vacuum, giving *5-chloro-2-m-nitrophenylpyrimidine*, m. p. 172—173° (Found: C, 51·3; H, 2·5; N, 17·9. $C_{10}H_6O_2N_3Cl$ requires C, 51·0; H, 2·5; N, 17·8%).

5-Chloro-2-p-nitrophenylpyrimidine. Prepared in a similar manner, the *5-chloro-2-p-nitrophenylpyrimidine* (yield, 35%) had m. p. 228° (Found: C, 51·0; H, 2·5; N, 17·8%).

Catalyst for Dehalogenation of Chloropyrimidines.—5% Palladised barium sulphate (Mozingo, *Org. Synth.*, **26**, 77) was used as the catalyst throughout.

Reduction of 2 : 5-Dichloropyrimidine in the Presence of Magnesium Oxide: Preparation of Pyrimidine.—The dichloro-compound (20·6 g.), magnesium oxide (6 g.), and catalyst (2 g.) in water (200 c.c.) were shaken with hydrogen at room temperature and pressure till 5·7 l. had been absorbed (*ca.* 12 hours). After removal of the catalyst the solution was treated with mercuric chloride solution containing enough hydrochloric acid to keep the pH at 3—5, and the precipitate collected, washed, and dried. Combined material (110 g.) from three such experiments was distilled with sodium sulphide (115 g. of nonahydrate), and the distillate saturated with potassium carbonate, giving an upper pyrimidine layer (24 c.c.) and a lower alkaline layer (108 c.c.). The latter was extracted with ether and the extract combined with the pyrimidine layer, dried ($CaSO_4$), and freed from ether. The residue was fractionally distilled through a small and efficient column (Dixon, *J. Soc. Chem. Ind.*, 1949, **68**, 299). Several fractions were collected in the b. p. range 121—123°; all solidified at room temperature (18°); the best fractions had m. p. 21·7°. The yield of substantially pure material was 42%. *Pyrimidine oxalate*, formed by mixing ethereal solutions of the two components, separated from alcohol as crystals, m. p. 160° (decomp.) and had the following light-absorption in water: $\lambda_{max.}$ 242·5 (ε, 2850); $\lambda_{min.}$ 221 (ε, 1015); $\lambda_{inflexion}$ 263—270 mμ. (ε, 415) (Found: C, 42·6; H, 3·7; N, 16·3. $C_4H_4N_2.C_2H_2O_4$ requires C, 42·4; H, 3·5; N, 16·5%).

Pyrimidine gave with aqueous solutions of copper sulphate and ammonium thiocyanate a green insoluble *complex*, analogous to that formed by pyridine [Found: N, 24·5. $(C_4H_4N_2)_2Cu(SCN)_2$ requires N, 24·7%). In solution in 0·1N-sodium hydroxide pyrimidine absorbed hydrogen in the presence of palladised barium sulphate only slowly; the rate of hydrogen uptake was increased 20-fold when the solution was made *ca.* 0·1N. with respect to hydrochloric acid.

In one experiment the hydrogenation of 2 : 5-dichloropyrimidine was stopped before the theoretical amount of hydrogen for replacement of both chlorine atoms had been absorbed. The product, isolated by fractional distillation, consisted of pyrimidine and a higher-boiling fraction from which a crystalline chloro-compound, m. p. 36·5°, was isolated. 2-Chloropyrimidine has m. p. 65° (see McOmie and Boarland, *Chem. and Ind.*, 1950, **31**, 602), so the compound must be *5-chloropyrimidine* (Found: C, 42·2; H, 2·4; N, 24·4. $C_4H_3N_2Cl$ requires C, 41·9; H, 2·6; N, 24·4%). It formed a white insoluble complex with mercuric chloride.

Attempted Reduction of 2 : 5-Dichloropyrimidine in the Presence of Alkali.—The dichloro-compound (1 g.), barium oxide (1 g.), and catalyst (1 g.) were shaken in methanol (35 c.c.) with hydrogen for 2 hours, but none was absorbed. Isolation in the usual way followed by sublimation in a vacuum gave *5-chloro-2-methoxypyrimidine*, m. p. 51·5° (Found: C, 41·9; H, 3·7; N, 19·6. $C_5H_5ON_2Cl$ requires C, 41·5; H, 3·5; N, 19·4%).

Reduction of 2 : 5-Dichloropyrimidine without Removal of Acid.—The dichloro-compound (5·2 g.), catalyst (1 g.), and water (100 c.c.) were shaken with hydrogen till no more was absorbed (uptake, 2·95 l.; 4 mols. of H_2 require 3·2 l.). The catalyst was removed, and the filtrate evaporated to small volume under reduced pressure, made alkaline, and extracted with ether. Treated with ethereal oxalic acid, the dried ethereal extract gave *tetrahydropyrimidine oxalate*, m. p. 151·5° (decomp.) (from alcohol), depressed on admixture with pyrimidine oxalate (Found: C, 41·5; H, 6·2; N, 16·1. $C_4H_8N_2.C_2H_2O_4$ requires C, 41·4; H, 5·8; N, 16·1%). The same material was obtained when the reduction was carried out in the presence of calcium carbonate.

Reduction of 4-Chloro-5-phenylpyrimidine.—The chloro-compound (0·5 g.; Davies and Piggott, *loc. cit.*) and catalyst (0·5 g.) were shaken in methanol (40 c.c.) containing 10% aqueous sodium hydroxide (1·5 c.c.) with hydrogen, of which 1·4 mols. were absorbed. The product was treated with picric acid, giving *5-phenylpyrimidine picrate*, m. p. 120° (from alcohol) (Found: C, 49·5; H, 3·2; N, 18·0. $C_{10}H_8N_2.C_6H_3O_7N_3$ requires C, 49·6; H, 2·9; N, 18·2%). Davies and Piggott record for 5-phenyltetrahydropyrimidine picrate m. p. 201—202°.

Reduction of 5-Chloro-2-phenylpyrimidine.—(*a*) The chloro-compound (5·52 g.), catalyst (1 g.), methanol (200 c.c.), and 10% aqueous sodium hydroxide (15 c.c.) were shaken together at 25° with hydrogen, of which 1 mol. was absorbed. The product was isolated in the usual way and sublimed in a vacuum, giving *2-phenylpyrimidine* (4·2 g.), m. p. 37—38° (Found: C, 76·9; H, 5·0; N, 18·3. $C_{10}H_8N_2$ requires C, 76·9; H, 5·1; N, 18·0%). Cherbuliez and Stavritch (*loc. cit.*) record m. p. 128° for a compound to which this structure was assigned.

(*b*) When the chloro-compound (0·84 g.), catalyst (1 g.), and methanol (20 c.c.) were shaken with hydrogen, 3 mols. were absorbed. Removal of catalyst and solvent, and crystallisation of the residue from alcohol, gave *1 : 4 : 5 : 6-tetrahydro-2-phenylpyrimidine hydrochloride*, m. p. 243·5° (Found: C, 60·6; H, 6·3; N, 14·3. $C_{10}H_{12}N_2.HCl$ requires C, 61·1; H, 6·6; N, 14·2%). The free base had m. p. 86—87°, in agreement with Aspinall (*loc. cit.*), and the picrate, m. p. 180°.

The free base (100 mg.) and platinised charcoal (Linstead's catalyst-d; *J.*, 1940, 1127) were heated together at 260—270° in a current of carbon dioxide for 3 hours and the product (70 mg.; m. p. 34—36°) extracted from the cooled mixture with ether. Sublimation in a vacuum gave 2-phenylpyrimidine, m. p. 36·5°, undepressed on admixture with material obtained by method (*a*) above.

Reduction of 4-Chloro-2-phenylpyrimidine.—The chloro-compound (0·5 g.), catalyst (0·5 g.), and methanol (25 c.c.) containing 10% aqueous sodium hydroxide (1·5 c.c.) were shaken together with hydrogen, of which 1 mol. was absorbed in 2 hours. Isolation in the usual way gave 2-phenylpyrimidine, m. p. 36—37°.

Reduction of 2-Chloro-4-phenylpyrimidine.—The chloro-compound (1·07 g.; prepared as described below) was reduced just as described for 4-chloro-2-phenylpyrimidine. Crystallised from aqueous

alcohol, the 4-phenylpyrimidine had m. p. 61—62°. We were unable to duplicate the m. p. 66—67° recorded for this compound by Ochiai and Kataoka (*loc. cit.*).

Reduction of 5-Chloro-2-nitrophenylpyrimidines.—These reductions were carried out in the presence of aqueous sodium hydroxide in methanol, as described for the parent 5-chloro-2-phenylpyrimidine. The products were obtained in almost quantitative yield and were purified by sublimation in a vacuum.

2-m-*Aminophenylpyrimidine* had m. p. 100° (Found: C, 69·7; H, 5·2; N, 24·9. $C_{10}H_9N_3$ requires C, 70·2; H, 5·3; N, 24·6%).

2-p-*Aminophenylpyrimidine* had m. p. 145—146° (Found: C, 70·4; H, 5·3; N, 24·5%).

2-Hydroxy-4-phenylpyrimidine.—The 2-amino-4-phenylpyrimidine required for this preparation was obtained by the following modification of the method used by Sprague *et al.* (*J. Amer. Chem. Soc.*, 1941, **63**, 3028), who obtained a yield of only 10%. Guanidine nitrate (3·5 g.), sodiobenzoylacetaldehyde (4 g.), and pyridine (15 c.c.) were heated together under reflux for 4 hours. The pyridine was removed under reduced pressure, a little sodium hydroxide solution added to the residue, the insoluble material collected and dissolved in dilute hydrochloric acid, and the filtered solution made alkaline. The amino-compound (1·7 g., 42%) was collected and after recrystallisation from dilute alcohol had m. p. 165°.

To a stirred solution of the amino-compound (13·4 g.) in 12% sulphuric acid (160 c.c.) containing a little octanol a solution of sodium nitrite (10 g.) in water (30 c.c.) was added slowly, whilst the temperature was kept below 10°. Stirring was then continued at room temperature for 6 hours, after which the solution was kept overnight, then filtered, and the filtrate adjusted to pH *ca.* 3, with concentrated aqueous ammonia. The precipitate, collected and recrystallised from alcohol, gave *2-hydroxy-4-phenylpyrimidine* (6 g.), m. p. 240—241° (Found: N, 16·0. $C_{10}H_8ON_2$ requires N, 16·3%). Addition of more ammonia to the filtrate of pH 3 precipitated unchanged starting material (6 g.).

2-Chloro-4-phenylpyrimidine.—The above hydroxy-compound (2·83 g.) and phosphoryl chloride (15 c.c.) were heated together under reflux for 1½ hours and the solution kept overnight and then poured on crushed ice. The solid was collected and the filtrate made alkaline with aqueous sodium carbonate and extracted with ether. The ether was removed, and the residue combined with the solid referred to above and sublimed in a vacuum. The chloro-compound (1·4 g., 45%) so obtained had m. p. 88·5—89·5°; Matsukawa and Ohta (*J. Pharm. Soc. Japan*, 1950, **70**, 134) give m. p. 87—88° for this compound.

Reaction between Pyrimidine and Diazotised p-*Nitroaniline.*—A solution of *p*-nitrophenyldiazonium chloride (6 c.c.; prepared from 0·93 g. of *p*-nitroaniline) was added slowly with stirring to pyrimidine (3·4 g.); the solution was then diluted to 100 c.c., and filtered, and sodium acetate (2 g.) added to the filtrate, which was stirred for 5 hours and then kept for a further 2 days. The brown precipitate (0·77 g.) was collected and dried, and a portion (0·513 g.) was dissolved in benzene and filtered through a column of aluminium oxide. Development of the chromatogram with benzene gave two substantially pure fractions; that less strongly adsorbed (90 mg.; m. p. 196—198°) was sublimed in a vacuum giving 2-p-*nitrophenylpyrimidine*, m. p. 198—199° (Found: C, 59·9; H, 3·4; N, 21·1. $C_{10}H_7O_2N_3$ requires C, 59·7; H, 3·5; N, 20·9%). That more strongly adsorbed (130 mg.; m. p. 180—183°) gave on vacuum sublimation 4-p-*nitrophenylpyrimidine*, m. p. 184° (Found: C, 59·6; H, 3·4; N, 21·0%).

Identification of 2-p-*Nitrophenylpyrimidine by Synthesis.*—2-*p*-Aminophenylpyrimidine (102 mg.; prepared as described earlier) was dissolved in alcohol and diazotised at 0° by treatment with a little concentrated sulphuric acid and then with amyl nitrite. The diazonium compound was precipitated with ether, collected, and washed with more ether. Meanwhile, solutions of copper sulphate (0·5 g. of $CuSO_4.5H_2O$) and sodium sulphite (0·25 g.) were mixed and the precipitate collected, washed, and made into a sludge with sodium nitrite (1 g.) and water (4 c.c.); to this sludge the diazonium sulphate was added and the mixture was kept overnight. The solids were then filtered off and extracted with acetone; evaporation of the extract gave a crude product (78 mg.; m. p. 192°) which was sublimed under reduced pressure. The 2-*p*-nitrophenylpyrimidine so obtained separated from alcohol as needles, m. p. 199°, undepressed on admixture with the material obtained from the Gomberg reaction above.

p-*Nitrobenzoylacetaldehyde.*—Benzene (70 c.c.) containing ethyl formate (12·4 g.) and *p*-nitroacetophenone (18·6 g.) was kept in contact with sodium wire (2·6 g.) which, after 1 week, had all reacted to give a black mass. This was collected, washed with ether, and treated with water; the mixture was filtered and the filtrate acidified. The precipitate was collected, dried, and extracted with hot light petroleum (b. p. 60—80°); when cooled the extract gave yellow needles of p-*nitrobenzoylacetaldehyde* (ω-*formyl*-p-*nitroacetophenone*) (6·3 g.), m. p. 98—99° (Found: C, 56·3; H, 3·3; N, 7·0. $C_9H_7O_4N$ requires C, 56·0; H, 3·3; N, 7·2%). Attempts to condense this material with guanidine were unsuccessful.

Identification of 4-p-*Nitrophenylpyrimidine.*—The nitro-compound (100 mg.) of m. p. 184° from the Gomberg reaction was hydrogenated in methanol (40 c.c.) in the presence of 5% palladised barium sulphate (0·5 g.); the product, isolated as usual, was sublimed in a vacuum, giving 4-*p*-aminophenylpyrimidine (80 mg.), m. p. 196°. A portion (60 mg.) was diazotised at 0° with concentrated hydrochloric acid (0·25 c.c.) and sodium nitrite [29·4 mg. in water (0·2 c.c.)], and the mixture was kept at 0° for 24 hours with 30% hypophosphorous acid (1 c.c.), and then extracted with ether. The ethereal extract was washed with aqueous sodium hydroxide and with water, then dried and evaporated. Sublimation of the residue in a vacuum gave 4-phenylpyrimidine, m. p. 61—62°, undepressed on admixture with material prepared as described earlier (Found: N, 18·5. Calc. for $C_{10}H_8N_2$: N, 18·0%).

5-Amino-2-phenylpyrimidine.—5-Nitro-2-phenylpyrimidine (2·11 g.; Hale and Brill, *J. Amer. Chem. Soc.*, 1912, **34**, 82) was hydrogenated in alcohol (200 c.c.) with palladised barium sulphate. The product was recrystallised from benzene, giving *5-amino-2-phenylpyrimidine* (90%), m. p. 90—91° (Found: C, 70·1; H, 5·1; N, 24·4. $C_{10}H_9N_3$ requires C, 70·3; H, 5·3; N, 24·5%).

Nitration of 2-Phenylpyrimidine.—2-Phenylpyrimidine (4·18 g.) and nitric acid (2 c.c.; *d* 1·42) were mixed, cooled, and treated with concentrated sulphuric acid (12 c.c.). The solution was heated at 100°

for ½ hour, cooled, and poured into water (75 c.c.); a yellow crystalline solid (4·5 g.; m. p. 130—136°) separated and was collected. Recrystallisation from alcohol gave 2-m-*nitrophenylpyrimidine* as needles, m. p. 140—141° (Found: C, 59·9; H, 3·7; N, 20·9. $C_{10}H_7O_2N_3$ requires C, 59·7; H, 3·5; N, 20·9%). The same material was isolated by chromatography of the crude product on aluminium oxide with benzene as solvent. A trace of material, m. p. 159—161°, was also obtained, but could not be examined more closely.

Identification of 2-m-*Nitrophenylpyrimidine.*—2-*m*-Aminophenylpyrimidine (102 mg.; prepared as already described) was converted into the corresponding nitro-compound in a way similar to that described above for 2-*p*-aminophenylpyrimidine. The *m*-nitro-compound so obtained had m. p. 140—141°, undepressed on admixture with the product of nitration of 2-phenylpyrimidine.

4 : 6-*Dimethyl*-2-m-*nitrophenylpyrimidine.*—Acetylacetone (0·78 g.), *m*-nitrobenzamidine hydrochloride (1·58 g.), potassium carbonate (2·1 g.), and water (7·5 c.c.) were warmed together till a clear solution resulted; this was then kept for 20 hours. The crystalline precipitate was collected, washed with dilute hydrochloric acid and with water, dried, and sublimed under reduced pressure, which gave the nitro-compound, m. p. 154—155°, undepressed on admixture with material obtained by nitrating 4 : 6-dimethyl-2-phenylpyrimidine as described by Ochiai *et al.* (*loc. cit.*) (Found: C, 62·6; H, 5·0; N, 18·3. Calc. for $C_{12}H_{11}O_2N_3$: C, 62·9; H, 4·8; N, 18·3%).

Nitration of 4-*Phenylpyrimidine.*—4-Phenylpyrimidine (0·34 g.) was nitrated as described for 2-phenylpyrimidine, and the cooled reaction mixture diluted with water and aqueous ammonia added to pH *ca.* 4. The precipitate (100 mg.; m. p. 115—118°) was collected; further addition of ammonia to the filtrate gave a second crop of material (0·26 g.; m. p. 80—100°) from which, however, no pure substance could be isolated. The first crop was dissolved in benzene and the solution filtered through a column of aluminium oxide. Development of the chromatogram with benzene–alcohol gave an effluent which was evaporated; the residue sublimed in a vacuum. The pure material so obtained had m. p. 121—121·5° and was 4-m-*nitrophenylpyrimidine* (Found: C, 60·1; H, 3·5; N, 20·6%).

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516. *Organic Fluorides. Part IX.* The Formation and Resolution of α-Hydroxy-α-trifluoromethylpropionic Acid.*

By R. A. DARRALL, F. SMITH, M. STACEY, and J. C. TATLOW.

The action of sodium cyanide on 1 : 1 : 1-trifluoroacetone afforded the corresponding racemic cyanohydrin which was converted into the racemic α-hydroxy-α-trifluoromethylpropionic acid. Certain derivatives of the latter product were made, and fractional crystallisation of its brucine salt enabled the *dextro*- and the *lævo*-acid to be isolated.

TRIFLUOROACETONE was first prepared by Swarts (*Bull. Acad. roy. Belg.*, 1927, **13**, 175) by the acid hydrolysis of trifluoroacetoacetic ester, which was obtained (*idem, ibid.*, 1926, **12**, 692) by condensation of ethyl trifluoroacetate with ethyl acetate in the presence of sodium ethoxide (cf. Henne, Newman, Quill, and Staniforth, *J. Amer. Chem. Soc.*, 1947, **69**, 1819; Haszeldine, Musgrave, Smith, and Turton, *J.*, 1951, 609). When this work was initiated little was known of the chemical properties of trifluoroacetone except that it formed a relatively stable hydrate and gave an oxime, a semicarbazone, and a bisulphite compound (Swarts, *loc. cit.*, 1927). Our interest in organic fluorides led us to a further study of the chemistry of trifluoroacetone and related fluoro-ketones.

This paper deals with the application of the cyanohydrin reaction to trifluoroacetone (Smith, *Ann. Reports*, 1947, **44**, 117). Other reactions of fluoro-ketones will form the subject of later communications. Treatment of trifluoroacetone with sodium cyanide according to the method of Welch and Clemo (*J.*, 1928, 2629) readily afforded the corresponding cyanohydrin, which was hygroscopic. Although this compound was more stable than acetone cyanohydrin, small amounts being distillable at atmospheric pressure with little or no decomposition, yet prolonged heating caused dissociation into trifluoroacetone and hydrogen cyanide. Treatment of the cyanohydrin with alcoholic ammonium sulphide gave α-hydroxy-α-trifluoromethyl(thiopropionamide) and this, on hydrolysis with dilute acid, readily gave the crystalline α-hydroxy-α-

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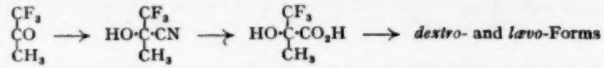
trifluoromethylpropionic acid. The latter was also obtained directly from the cyanohydrin by graded hydrolysis, in which reaction the amide was isolated as an intermediate.



The α-hydroxy-α-trifluoromethylpropionic acid was hygroscopic; its aqueous solutions were strongly acidic ($K = 1{\cdot}3 \times 10^{-3}$) and gave a yellow colour with ferric chloride. Potentiometric titrations of this acid and of the nitrile with sodium hydrogen carbonate and with methanolic sodium methoxide showed that the hydroxy-group had no acidic properties. It is of interest that 1 : 1 : 1-trifluoropropan-2-ol has acidic properties (Swarts, *Bull. Soc. chim. Belg.*, 1929, **38**, 99) and that, although dimethyltrifluoromethylcarbinol is not titratable with dilute alkalis, yet it does form an alcoholate with anhydrous potassium carbonate (*idem*, *ibid.*, 1927, **36**, 191). It would thus appear that in the case of α-hydroxy-α-trifluoromethylpropionic acid the trifluoromethyl group exerts its influence on the carboxyl group and not on the hydroxyl group.

Esterification of α-hydroxy-α-trifluoromethylpropionic acid with methyl alcohol proceeded normally in the presence of a sulphuric acid catalyst to give the methyl ester, which afforded α-hydroxy-α-trifluoromethylpropionamide when treated with methanolic ammonia. There was no evidence that hemilactides or lactides were formed at all readily by heating the acid, which could be sublimed and distilled (b. p. 189°) unchanged. α-Hydroxy*iso*butyric acid when heated to 190° gives the corresponding lactide, together with methacrylic acid and acetone (Blaise and Bagard, *Ann. Chim. Phys.*, 1907, **11**, 111).

By treatment with acetic anhydride and fused sodium acetate, both trifluoroacetone cyanohydrin and methyl α-hydroxy-α-trifluoromethylpropionate afforded acetates, there being no evidence of the formation of any unsaturated products. Under similar conditions γγγ-trichloro-β-hydroxybutyric acid undergoes dehydration and yields γγγ-trichlorocrotonic acid (Auwers and Schmidt, *Ber.*, 1913, **46**, 487). Concentrated sulphuric acid reacted with the cyanohydrin to give α-hydroxy-α-trifluoromethylpropionamide; by the addition of water or of methyl alcohol to the reaction mixture followed by heating, the amide could be converted *in situ* into the acid or the methyl ester, respectively. By reactions similar to these, acetone cyanohydrin may be converted into derivatives of methacrylic acid (Crawford, B.P. 405,699, 427,810). Unsaturated products could not be isolated after reaction of trifluoroacetone cyanohydrin or of methyl α-hydroxy-α-trifluoromethylpropionate with phosphoric oxide.

Thus, as a result of the electronegative effect of the CF_3 group, the hydroxy-group of α-hydroxy-α-trifluoromethylpropionic acid and of its derivatives is more resistant to replacement than are orthodox tertiary alcoholic groups. The hydroxy-group of dimethyltrifluoromethylcarbinol behaves similarly, but Swarts (*loc. cit.*) succeeded in dehydrating this alcohol with phosphorus pentabromide, a reagent which might well convert derivatives of α-hydroxy-α-trifluoromethylpropionic acid into trifluoromethylacrylic acid derivatives.

Since this work was done Dickey (U.S.P. 2,472,812; *Chem. Abs.*, 1949, **43**, 8398) has reported the preparation of α-trifluoromethylacrylamide by treatment of trifluoroacetone cyanohydrin with concentrated sulphuric acid, and of alkyl α-trifluoromethylacrylates from the cyanohydrin and alkyl hydrogen sulphates.

The reaction between trifluoroacetone and sodium cyanide introduces asymmetry into the molecule. In order to determine the effect of a CF_3 group on optical activity, the racemic α-hydroxy-α-trifluoromethylpropionic acid was resolved into its enantiomorphs by fractional crystallisation of the diastereoisomeric brucine salts. Decomposition of the latter afforded the corresponding optically active acids which showed $[\alpha]_D$ $+11{\cdot}5°$ and $-11{\cdot}5°$. It is evident that the CF_3 group does not greatly affect the optical activity. As far as we are aware this is the first optically active acid to be isolated having a perfluoro-substituent on an asymmetric carbon atom.

Experimental.

Preparation of 1 : 1 : 1-*Trifluoroacetone Cyanohydrin.*—(a) 1 : 1 : 1-*Trifluoroacetone* (Swarts, *Bull. Acad. roy. Belg.*, 1927, **13**, 175). Ethyl trifluoroacetate (227·0 g., 15% excess) was treated with sodium ethoxide (94·7 g.) in dry ether (800 c.c.); ethyl acetate (148·7 g., 20% excess) was then added, and the solution was refluxed for 60 hours. The ether and excess of esters were removed by distillation *in vacuo*. To the sodio-derivative of trifluoroacetoacetic ester thus obtained, ice (220 g.) was added, followed by an ice-cold mixture of concentrated sulphuric acid (170 c.c.) and water (780 c.c.). The mixture was heated at 80—100° for 4 hours, and the trifluoroacetone (b. p. 22°) evolved was used directly for the next stage.

A portion of the trifluoroacetone gave in the usual way a semicarbazone, m. p. 127° (Found: C,

28·4; H, 3·3; F, 33·3. Calc. for $C_4H_4ON_3F_3$: C, 28·4; H, 3·6; F, 33·7%). Swarts (*loc. cit.*) recorded m. p. 127° for this compound.

(b) 1 : 1 : 1-*Trifluoroacetone cyanohydrin*. The trifluoroacetone from the previous experiment was passed directly into a stirred solution of sodium cyanide (63·6 g.) in water (272 c.c.) cooled to 0°. When all the trifluoroacetone had been absorbed in the solution, 6N-sulphuric acid (455 g.) cooled to 0° was added slowly, with stirring, and the solution was kept overnight at room temperature. The reaction mixture was then extracted with ether. The combined ethereal extracts (1 l.) were dried (Na_2SO_4) and filtered, and the ether was removed by distillation through a short column. The residue was redried (Na_2SO_4), filtered, and distilled under diminished pressure into a receiver cooled in ice and salt. The distillate was mixed with an equal volume of dry ether and again dried (Na_2SO_4), the ether was removed by distillation, and the residue was distilled to give 1 : 1 : 1-*trifluoroacetone cyanohydrin* (139·0 g.), b. p. 68—69°/40 mm., 138—139°/760 mm., n_D^{20} 1·3385 (Found : C, 34·5; H, 3·1; F, 40·9. $C_4H_4ONF_3$ requires C, 34·55; H, 2·9; F, 41·0%). This cyanohydrin was hygroscopic. Potentiometric titration of it in 91% aqueous methanol with 0·54N-sodium methoxide in 91% aqueous methanol showed that it was a neutral substance.

Treatment of the cyanohydrin (5·00 g.) with a mixture of acetic anhydride (14·0 c.c.) and fused sodium acetate (1·50 g.) at 100—105° for 3 hours, followed by pouring of the reaction mixture into water, and extraction with ether, afforded the *acetate* (3·57 g.), b. p. 150—152°, n_D^{18} 1·355 (Found : F, 31·7. $C_6H_6O_2NF_3$ requires F, 31·5%).

Thioamide of α-Hydroxy-α-trifluoromethylpropionic Acid.—Trifluoroacetone cyanohydrin (20·0 g.) was dissolved in ethyl alcohol (44·0 c.c.) and a saturated solution of ammonia in ethyl alcohol (44·0 c.c.) was added. The mixture was cooled in ice, and dry hydrogen sulphide was passed in until a copious precipitate had appeared. After being kept for 18 hours and the precipitate had redissolved, the mixture was concentrated *in vacuo* at 100°. Distillation of the residue yielded the *thioamide* as a pale yellow oil (20·0 g.), b. p. 70—76°/0·9 mm., which crystallised spontaneously on cooling; it had m. p. 52° before or after crystallisation from carbon tetrachloride (Found : C, 27·8; H, 3·6. $C_4H_6ONSF_3$ requires C, 27·7; H, 3·5%).

Amide of α-Hydroxy-α-trifluoromethylpropionic Acid.—The cyanohydrin (10·0 g.) was added dropwise to concentrated sulphuric acid (5·9 c.c.), with mechanical stirring. The temperature of the reaction mixture rose spontaneously to 70—80°, and was kept there by regulation of the rate of addition of the cyanohydrin. When the addition was complete the white semi-solid mass was heated at 115—120° for 15 minutes, and was then cooled in an ice-bath whilst water (30 c.c.) was added. The clear solution was kept for 48 hours at 0°; the crystalline precipitate of amide which had formed was combined with that extracted from the aqueous solution with ether to give, after crystallisation from benzene–acetone, *α-hydroxy-α-trifluoromethylpropionamide* (5·3 g.), m. p. 142° (Found : C, 31·0; H, 3·7; F, 36·4. $C_4H_6O_2NF_3$ requires C, 30·6; H, 3·8; F, 36·3%).

The aqueous solution, after the ether-extraction, was heated at 100° for 7 hours and was then extracted exhaustively with ether. The ethereal extracts were dried (Na_2SO_4), filtered, and distilled; the residue was sublimed to give α-hydroxy-α-trifluoromethylpropionic acid (3·05 g.), m. p. and mixed m. p. 87·5°.

Preparation of α-Hydroxy-α-trifluoromethylpropionic Acid.—(*a*) The thioamide (22·15 g.) (see above) was heated at 100° with 20% hydrochloric acid (110 c.c.) for 18 hours, and the solution, after cooling, was extracted exhaustively with ether. The extract was dried (Na_2SO_4), the ether distilled, and the residue sublimed at 45—70° (bath-temp.)/0·25 mm. Resublimation of the product afforded *α-hydroxy-α-trifluoromethylpropionic acid* (17·85 g.), m. p. 88°, b. p. 189° [Found : C, 30·5; H, 3·2; F, 36·2%; *M* (by McCoy's method with benzene as solvent), 163; *E* (by potentiometric titration either in water or in 91% aqueous methanol), 159. $C_4H_5O_3F_3$ requires C, 30·4; H, 3·2; F, 36·1%; *M*, 158; *E*, 158]. The acid was deliquescent and gave a yellow colour with ferric chloride.

(*b*) The reaction described above for the preparation of α-hydroxy-α-trifluoromethylpropionamide was repeated with the same quantities; after the addition of water (31·5 c.c.), the mixture was heated at 100° for 6½ hours. Exhaustive extraction of the clear solution with ether, followed by removal of solvent as above, gave, after purification by sublimation, α-hydroxy-α-trifluoromethylpropionic acid (10·4 g.), m. p. and mixed m. p. 88°.

Methyl α-Hydroxy-α-trifluoromethylpropionate.—α-Hydroxy-α-trifluoromethylpropionic acid (33·2 g.) was boiled under reflux for 15 hours with magnesium-dried methyl alcohol (16·0 c.c.) and concentrated sulphuric acid (0·5 c.c.). The alcohol was then removed by distillation through a short column, and the residue was poured into ice-water. The aqueous phase was extracted three times with ether, and the extracts were washed with water, dried ($MgSO_4$), and freed from ether. Distillation of the residue gave *methyl α-hydroxy-α-trifluoromethylpropionate* (27·3 g.), b. p. 127—129°, n_D^{14} 1·358 (Found : C, 34·9; H, 4·2; F, 33·3. $C_5H_7O_3F_3$ requires C, 34·9; H, 4·1; F, 33·1%). From the aqueous layers there was obtained unchanged α-hydroxy-α-trifluoromethylpropionic acid (5·2 g.), m. p. and mixed m. p. 88°. The methyl ester could be distilled unchanged over sulphuric acid and over phosphoric oxide.

Methyl α-hydroxy-α-trifluoromethylpropionate (5·00 g.) was heated for 2 hours at 100° with a mixture of acetic anhydride (5·0 c.c.) and fused sodium acetate (0·48 g.). The mixture, after being poured into water and extracted with ether in the usual way, gave the *acetate* (3·07 g.), b. p. 155—156°, n_D^{18} 1·368 (Found : F, 26·5. $C_7H_9O_4F_3$ requires F, 26·6%).

When a solution of the methyl ester (0·950 g.) in methyl alcohol (10·0 c.c.), saturated at 0° with ammonia, was kept for 18 hours at room temperature, distillation of the solution left a residue which, on recrystallisation from benzene–acetone, afforded α-hydroxy-α-trifluoromethylpropionamide (0·715 g.), m. p. 142° alone or on admixture with a specimen prepared from the cyanohydrin (see above).

Optical Resolution of α-Hydroxy-α-trifluoromethylpropionic Acid.—A solution of the racemic fluoro-acid (5·28 g.) in methyl alcohol was mixed with a solution of brucine (15·59 g.) in methyl alcohol (total volume 350 c.c.), and the mixture was heated to boiling, filtered, and set aside until crystallisation appeared to be complete (3 days). This brucine salt was recrystallised (five times) from methanol until the rotation was constant. The product obtained (1·17 g.) consisted of large needles which showed $[\alpha]_D^{20}$ −30·3° (*c*, 1·0 in water). A portion of this salt (0·583 g.) was treated with sodium hydroxide solution, the precipitated brucine was removed by filtration, the solution was acidified, and the product was extracted with ether and purified as already described. The *lævo*-acid thus obtained (0·124 g.) had $[\alpha]_D^{16}$ −11·3° (*c*, 5·0 in water).

The mother-liquors from which the original precipitate was obtained were concentrated, and the more soluble isomer of the brucine salt was purified by systematic fractional crystallisation until it showed a constant negative rotation. The pure diastereoisomer (0·434 g.) thus produced had $[\alpha]_D^{20}$ −17·4° (*c*, 1·0 in water). Treatment of this brucine salt as above gave the *dextro*-form of the acid (0·092 g.), which showed $[\alpha]_D^{20}$ +11·4° (*c*, 3·7 in water).

The authors acknowledge their gratitude to the late Professor Sir Norman Haworth, F.R.S., for his interest in this work, and to Dr. L. M. Turton for carrying out the molecular-weight determination. The work was carried out with support from the Ministry of Supply and thanks are due to the Chief Scientific Officer for permission to publish it.

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517. *The Esterification of Unstable Alcohols.*

By J. A. MILLS.

Poor yields of esters from the reaction of certain alcohols with acid chlorides in pyridine are probably due to decompositions catalysed by the pyridinium ion. Good yields may be obtained by avoiding an excess of pyridine and working in a solvent which precipitates pyridine hydrochloride as it is formed. The method is illustrated by the esterification of (±)-4-methylpent-3-en-2-ol with several acid chlorides.

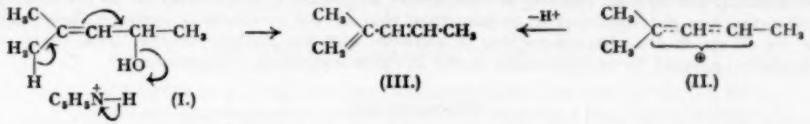
THE esterification of alcohols by reaction with the appropriate acid chlorides, pyridine being used as solvent and hydrogen chloride acceptor, sometimes gives unsatisfactory results. Blumann and Zeitschel (*Ber.*, 1913, **46**, 1178) reported a 50% yield of benzoate from verbenol, Read and Walker (*J.*, 1934, 308) only 13% of 3 : 5-dinitrobenzoate from a piperitol fraction, and Grubb and Read (*ibid.*, p. 242) did not isolate any ester from the product of the Meerwein–Ponndorf reduction of pulegone, although work in these laboratories (unpublished) indicates that the reduction product contains a considerable amount of pulegol. Attempts to prepare a *p*-nitrobenzoate from 4-methylpent-3-en-2-ol failed completely (Kenyon and Young, *J.*, 1940, 1547).

The alcohols which have given poor yields are all secondary alcohols containing an allylic system, being αγγ-trialkyl- or αβγγ-tetra-alkyl-allyl alcohols. These highly alkylated structures are known to be favourable for acid-catalysed reactions, such as dehydration or anionotropic rearrangement, and the acid-catalysed dehydration often produces dienes corresponding to "abnormal" or 1 : 4-elimination (Bacon and Farmer, *J.*, 1937, 1065; Dupont and Darmon, *Bull. Soc. chim.*, 1939, **6**, 1208; Kenyon and Young, *loc. cit.*; Read and Walker, *loc. cit.*). It is probable that esters of such alcohols will also undergo acid-catalysed decomposition to dienes at about the same rate as do the free alcohols (cf. Braude, *J.*, 1948, 794, on anionotropic rearrangements of alcohols and esters).

A probable explanation of the poor yields of esters in the usual method of preparation is the destruction of alcohol or ester, or both, by an acid-catalysed reaction initiated by the pyridinium ion which is present because of the appreciable solubility of pyridine hydrochloride in pyridine. The warming which is often applied in the conventional procedure is also an unfavourable factor. It is unimportant for the present purpose whether the diene formation from 4-methylpent-3-en-2-ol, for example, is truly a concerted 1 : 4-elimination, such as (I), or proceeds in two steps with the mesomeric carbonium ion (II) as intermediate; either route will lead to the diene (III) as the principal product, and removal of the pyridinium ion from solutions of the alcohol or ester should suppress the formation of (III).

These considerations, with an earlier observation (Gillespie, Macbeth, and Mills, *J.*, 1948, 996) that yields of nitrobenzoic esters from 4-*iso*propyl*cyclo*hex-2-en-1-ol were considerably

improved when the reaction was carried out in benzene with only a moderate excess of pyridine, have suggested a successful general procedure for the esterification of such unstable alcohols. The alcohol and acid chloride are dissolved separately in benzene or light petroleum, according



(I.) (III.) (II.)

to their solubility, and allowed to react in the presence of a slight excess of pyridine. Pyridine hydrochloride crystallises out as it is formed, and the non-polar solvent suppresses the ionisation of any which remains in solution. Heating is avoided, and mineral acids are not used in working up the product.

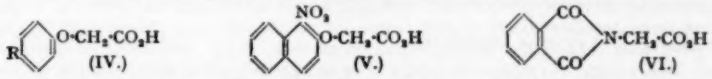
The esterification of 4-methylpent-3-en-2-ol by various acid chlorides was selected as a test case, and in every instance good yields of ester were obtained. Once isolated, the esters were quite stable, and could be recrystallised from polar solvents; the instability mentioned by Kenyon and Young (*loc. cit.*) should be ascribed more to the conditions of preparation than to the nature of the esters. The procedure described has been used successfully by other workers in these laboratories for the isolation of terpene alcohols, and the esterification of piperitol has been reported by Barnes, Jackman, and Macbeth (*J.*, 1951, 1848), with a preliminary description of the method used. Pulegol has also been esterified, but the investigation is incomplete. The isolation of the more unstable types of terpene alcohol now seems to be possible on a scale suitable for detailed examination.

No novelty is claimed for the use of an inert diluent in esterifications with acid chlorides (see, *e.g.*, Reichstein, *Helv. Chim. Acta*, 1926, **9**, 799, 803; Friedman and Seligman, *J. Amer. Chem. Soc.*, 1950, **72**, 624), but it may not be generally realised that in certain cases it is essential to use this modification. In general, it is best to avoid the use of excess of pyridine as solvent in any reactions with acid chlorides if the primary product is susceptible to rearrangement, or to an exchange reaction with chloride ion (*e.g.*, some sulphonic esters).

The method described may be combined with the procedure for preparation of acid chlorides in solution (Human and Mills, *J.*, 1949, S 77; *Nature*, 1946, **158**, 877); the acid is allowed to react with one mole each of thionyl chloride and pyridine, and the resulting solution of acid chloride is added to a solution of the alcohol containing an additional one mole of pyridine. This provides a method for esterifying labile alcohols with labile acid chlorides.

Kenyon and Young's work (*loc. cit.*) on 4-methylpent-3-en-2-ol provides additional evidence that acid-catalysed decomposition is the main factor preventing successful esterification. Although the *p*-nitrobenzoate could not be made, these authors successfully esterified the alcohol with acetic anhydride and with *p*-diphenylyl *iso*cyanate; these reagents are regarded as powerful dehydrating agents, but strong acidity is not developed during esterifications with them. Kenyon and Young also found that a hydrogen phthalate could be made from the alcohol through the potassium derivative, but the free acid ester soon decomposed spontaneously. It is improbable that the readily-dehydrated alcohols mentioned above could be successfully purified through the hydrogen phthalates (cf. Blumann and Zeitschel, *loc. cit.*), and the (−)-pulegol which Paolini claimed to have isolated in this way (*Atti R. Accad. Lincei*, 1919, **28**, II, 190, 236) may well have some other structure.

p-Nitrobenzoates and 3 : 5-dinitrobenzoates of allylic alcohols usually decompose on being heated to temperatures not much above 100°, and this thermal instability is a disadvantage in the usual procedure for isolating terpene alcohols, as steam-distillation of the ester to remove terpenes may cause serious losses of ester from thermal decomposition and from the action of the acids produced. By conducting the steam-distillation in the presence of a little sodium carbonate the autocatalytic effect of free acid is prevented, but the thermal decomposition of the crude esters may still occur. It was thought that esters having greater thermal stability



(IV.) (V.) (VI.)

might be obtained from acids which differed from *p*-nitro- and 3 : 5-dinitro-benzoic acids in having the carboxyl group isolated from electronic interaction with an aromatic nucleus carrying strongly electronegative groups, but the results obtained with several substituted acetic acids

were not encouraging. *p*-Nitrophenoxyacetic (IV; R = NO_2), *p*-phenylazophenoxyacetic (IV; R = N:N·C_6H_5), 1-nitro-2-naphthoxyacetic (V), and phthalimidoacetic (VI) acids could all be converted into chlorides and thence into suitably crystalline esters with 4-methylpent-3-en-2-ol, but the thermal stability of these esters, as judged by their behaviour in the melting-point tube, was not significantly greater than that of the nitrobenzoic esters. These acids may be of use in the characterisation of alcohols, and the *p*-phenylazophenoxyacetates are sufficiently coloured to be potentially useful in chromatographic separations.

Experimental.

(±)-*4-Methylpent-3-en-2-ol.*—Commercial mesityl oxide was distilled through a 100-cm. column of single-turn Fenske helices and the fraction with b. p. 128·5—129° was used. The apparatus and general procedure for the Meerwein–Ponndorf reduction have been described previously (Macbeth and Mills, *J.*, 1949, 2646). From mesityl oxide (86 g.), dissolved in dry *iso*propanol (200 ml.) and added dropwise during 4·5 hours to a refluxing solution of distilled aluminium *iso*propoxide (104 g.) in *iso*propanol (600 ml.) and heated for a further 2 hours until the 2 : 4-dinitrophenylhydrazine test for ketone became negative, a crude product (75 g.) was obtained with b. p. 55—58°/15 mm. This was redistilled through the 100-cm. column, and the fraction (60 g.) with constant b. p. of 56·5°/15 mm. was used for the esterifications. The alcohol remained colourless when stored in a brown-glass bottle for a year.

Acid Chlorides.—*p*-Nitrophenoxyacetic acid (Fritzsche, *J. pr. Chem.*, 1879, **20**, 267), *p*-phenylazophenoxyacetic acid (Mai and Schwabacher, *Ber.*, 1901, **34**, 3936), and 1-nitro-2-naphthoxyacetic acid (Lees and Shedden, *J.*, 1903, **83**, 750) were made by published procedures.

Each of the foregoing acids was converted smoothly into the chloride by warming it with pure thionyl chloride (3—5 moles) on the steam-bath for 1—2 hours. After removal of thionyl chloride under reduced pressure, followed by the evaporation of small portions of dry carbon tetrachloride, the *p*-nitrophenoxyacetyl chloride (Jacobs and Heidelberger, *J. Amer. Chem. Soc.*, 1917, **39**, 2418) was recrystallised from light petroleum, and the 1-nitro-2-naphthoxyacetyl chloride (Spitzer, *Ber.*, 1901, **34**, 3191) from carbon tetrachloride. The latter chloride crystallised directly from the excess of thionyl chloride in large crystals of high purity, but was recrystallised before use.

p-Phenylazophenoxyacetyl chloride is new, but was not obtained analytically pure. It crystallised from carbon tetrachloride containing light petroleum as small orange scales, m. p. 88—90°, but contained traces of anhydride or free acid which could not readily be removed. This material was quite suitable for esterifications; when added to absolute ethanol containing pyridine, it gave the known ethyl ester as shining orange scales, m. p. 70—71°; Mai and Schwabacher described this ester as greenish crystals with m. p. 70°.

Procedure for Esterification.—The esterification of 4-methylpent-3-en-2-ol with *p*-nitrobenzoyl chloride is typical of the procedure followed for the other esters. The chloride (0·02 mole), dissolved in dry benzene (25 ml.), was added to a stirred ice-cold solution of the alcohol (0·02 mole) and pyridine (0·022 mole) in light petroleum (40 ml.; b. p. 60—90°), and the mixture placed in the refrigerator overnight. Crystalline pyridine hydrochloride separated at once. The filtered solution of ester was washed several times with 1% aqueous sodium carbonate solution, then several times with water, and dried ($MgSO_4$). Evaporation of the solvent under reduced pressure left the ester as yellowish needles (82% yield). Recrystallisation from light petroleum (b. p. 60—90°) gave the pure ester with the properties recorded in the table.

Esters of (±)-4-methylpent-3-en-2-ol.

Ester.	Form.	M. p.	Formula.	Found, %. C.	Found, %. H.	Required, %. C.	Required, %. H.
p-Nitrobenzoate	Needles	71—72°	$C_{12}H_{13}O_4N$	62·9	5·8	62·7	6·1
p-Nitrophenoxyacetate	Scales	41—42	$C_{14}H_{17}O_5N$	60·3	6·3	60·2	6·1
1-Nitro-2-naphthoxyacetate	Plates	90—91	$C_{18}H_{19}O_5N$	65·9	6·1	65·6	5·8
p-Phenylazophenoxyacetate	Scales	59—60	$C_{20}H_{22}O_3N_2$	70·7	6·5	71·0	6·6

The other esters recorded were obtained in similar yields. The 3 : 5-dinitrobenzoate (Macbeth and Mills, *loc. cit.*) and phthalimidoacetate (Barnes, Jackman, and Macbeth, *loc. cit.*), prepared by this method, have already been described. All could be recrystallised without decomposition from light petroleum or aqueous methanol, and gave sharp, reproducible m. p.s, but when heated above the m. p. in capillary tubes they decomposed suddenly between 110° and 150°. The decomposition temperature varied somewhat with different samples of ester, but the phthalimidoacetate was the most stable, and the 3 : 5-dinitrobenzoate the least stable to heat. The *p*-phenylazophenoxyacetate was orange, and the other esters faintly yellow.

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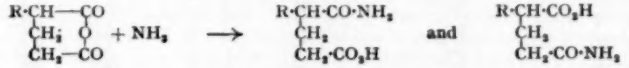
518. *The Influence of Substituent Groups on Ring Scission. Part I. The Ring Scission of Some Unsymmetrically Substituted Glutaric Anhydrides.*

By John C. Roberts and Keith Selby.

A method, employing partition chromatography, has been developed for the quantitative separation of the mixture of isomeric amic acids obtained when an unsymmetrically substituted glutaric anhydride reacts with ammonia. α-Phenylglutaric anhydride, dissolved in dioxan, yields at 20° with ammonia 63 ± 2% of the α-amic acid. Under the same conditions α-*p*-nitrophenylglutaric anhydride gives 46 ± 2% of the α-isomer. Five new arylglutaramic acids have been synthesised by unambiguous methods. The K_a' values for four arylglutaramic acids have been determined.

When an unsymmetrically substituted cyclic acid anhydride reacts with an alcohol, ammonia, or a substituted ammonia, two isomeric products are possible. For example, an α-substituted glutaric anhydride and ammonia may yield both the α- and the γ-amic acid :



Many examples of this type of reaction have been investigated, but in no case have both of the possible isomers been isolated quantitatively, and it should be noted that, at least in some cases, differences in the ease of isolation of the two products may lead to a false concept of the proportion of the isomers originally present. Anschütz *et al.* (*Annalen*, 1907, **354**, 117) have stated that, in the case of phenylsuccinic anhydride, the amino- or substituted amino-residue attaches itself to the carbonyl group corresponding to the " weaker " carboxyl group in the parent dicarboxylic acid, *i.e.*, to the carboxyl group further removed from the phenyl substituent. Unfortunately, these authors have not recorded the yields of the amic or substituted amic acids obtained, but have merely stated that the reaction products were homogeneous. King *et al.* (*J.*, 1949, 3315; 1951, 243) have shown that phthaloylglutamic anhydride possesses the remarkable property of giving very high yields (74—91%, crude products) of γ-derivatives when the ring is opened by ammonia, amino-compounds, or alcohols. However, the evidence available indicates that in most cases a mixture of isomers is obtained. For example, Le Quesne and Young (*J.*, 1950, 1954) have shown that *N*-carbobenzyloxyglutamic anhydride, with a variety of reagents, furnishes mixtures of α- and γ-derivatives (for further examples see Melville, *Biochem. J.*, 1935, **29**, 179; Bergmann, Zervas, and Fruton, *J. Biol. Chem.*, 1936, **115**, 606; Barry and Twomey, *Proc. Roy. Irish Acad.*, 1947, **51**, *B*, 152; Boothe *et al.*, *J. Amer. Chem. Soc.*, 1949, **71**, 2310; Roberts and Shaw, *J.*, 1950, 2842.)

There appears to be little doubt that the nature of the group which is unsymmetrically substituted in the anhydride ring markedly influences the composition of the mixture of isomers produced on ring scission. In order to obtain more precise information on this point we investigated quantitatively the reactions between ammonia and some glutaric anhydrides containing simple substituents in the α-position. This paper records our results with α-phenyl-, α-*p*-nitrophenyl-, and α-*p*-benzamidophenyl-glutaric anhydrides.

It became necessary to develop a method for the quantitative separation of the isomeric amic acids produced. Since they contained the same functional groups, it was considered that the ordinary process of partition chromatography (using water and an organic solvent) would not be successful, as such compounds would have almost identical distribution coefficients (Martin, *Ann. Reports*, 1948, **45**, 269). However, it appeared probable that the two isomers would differ in acidic strength, owing to the different relative positions of the substituent group and the carboxyl group, and this led us to expect that a special type of chromatographic analysis might be successful. The use of an immobile aqueous phase of constant pH has been shown to be advantageous in the separation of mixtures of bases or of acids (Craig, Golumbic, Mighton, and Titus, *J. Biol. Chem.*, 1945, **161**, 321; Moyle, Baldwin, and Scarisbrick, *Biochem. J.*, 1948, **43**, 308; Roberts and Selby, *J.*, 1949, 2785). Eventually we found that an efficient separation of the two amic acids, produced either from α-phenyl- or from α-*p*-nitrophenyl-

glutaric anhydride, could be obtained by using an immobile aqueous phase of pH 7·76 and a mobile phase of butanol–chloroform. It was not possible to obtain a satisfactory chromatogram with the *p*-benzamidophenylglutaramic acids because of their adverse solubility properties.

Further, it became necessary to devise an unequivocal synthesis for each amic acid and to make a prior determination of their chromatographic behaviour. γ-Carbamyl-α-phenylbutyric acid was efficiently synthesised by the following route:

$$Ph{\cdot}CH(CO_2Et)_2 \xrightarrow{CH[CH_2]_2{\cdot}CN} Ph{\cdot}C(CO_2Et)_2{\cdot}[CH_2]_2{\cdot}CN \xrightarrow[-CO_2]{\text{hydrolysis,}} Ph{\cdot}CH(CO_2H){\cdot}[CH_2]_2{\cdot}CN \xrightarrow[(H_2SO_4)]{H_2O} Ph{\cdot}CH(CO_2H){\cdot}[CH_2]_2{\cdot}CO{\cdot}NH_2$$

The isomeric γ-carbamyl-γ-phenylbutyric acid was obtained by the method of Wideqvist (*Svensk Kem. Tids.*, 1942, **54**, 34), who records m. p. 168°. However, the acid we obtained

FIG. 1.

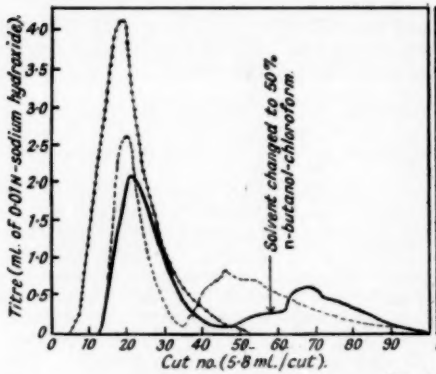


FIG. 2.

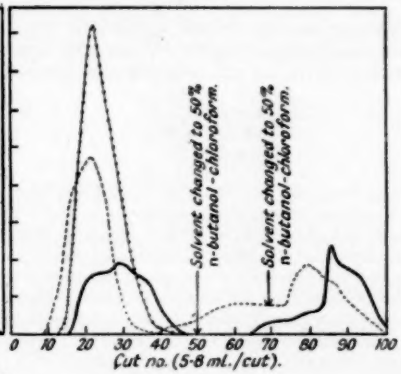


FIG. 1.

Immobile phase: 2M-*phosphate buffer solution*, pH 7·76 (*glass electrode*). *Mobile phase:* 30% (v/v) n-*butanol in chloroform at start of each run.* · — · — · — · — · *γ-Carbamyl-γ-phenylbutyric acid* (147 *mg.*). — — — — *A mixture of γ-carbamyl-γ-phenylbutyric acid* (51 *mg.*; $K_a' = 3{\cdot}63 \times 10^{-5}$ *at* 25°) *and γ-carbamyl-α-phenylbutyric acid* (51 *mg.*; $K_a' = 8{\cdot}32 \times 10^{-6}$ *at* 25°). ——— *Unknown mixture of phenylglutaramic acids* (101·5 *mg.*) *obtained by reaction of ammonia with α-phenylglutaric anhydride in dry dioxan at* 20°. *Proportion of γ-carbamyl-γ-phenylbutyric acid found* = 63 ± 2%.

FIG. 2.

Immobile phase: 2M-*phosphate buffer solution*, pH 7·76 (*glass electrode*). *Mobile phase:* 30% (v/v) n-*butanol in chloroform at start of each run.* · — · — · — · *γ-Carbamyl-γ-p-nitrophenylbutyric acid* (127 *mg.*). — — — — *A mixture of γ-carbamyl-γ-p-nitrophenylbutyric acid* (86 *mg.*; $K_a' = 3{\cdot}47 \times 10^{-5}$ *at* 25°) *and γ-carbamyl-α-p-nitrophenylbutyric acid* (82 *mg.*; $K_a' = 2{\cdot}34 \times 10^{-4}$ *at* 25°). ——— *Unknown mixture of* p-*nitrophenylglutaramic acids* (101 *mg.*) *obtained by reaction of ammonia with* α-p-*nitrophenylglutaric anhydride in dry dioxan at* 20°. *Proportion of γ-carbamyl-γ-p-nitrophenylbutyric acid found* = 46 ± 2%.

had m. p. 180° when prepared either by this method or by the alternative method, involving a chromatographic separation, from α-phenylglutaric anhydride and ammonia. The α-*p*-nitrophenyl- and α-*p*-benzamidophenyl-glutaric anhydrides and the corresponding α- and γ-amic acids were also synthesised (see below).

The appropriate anhydride, dissolved in dry dioxan, was treated with ammonia at 20°. The mixture of the free amic acids was isolated quantitatively from the ammonium salts produced and was analysed chromatographically. Each acid was isolated and was identified by comparison with its synthetic analogue.

α-Phenylglutaric anhydride yielded a mixture containing 63 ± 2% of γ-carbamyl-γ-phenylbutyric acid and 37 ± 2% of γ-carbamyl-α-phenylbutyric acid (Fig. 1); α-*p*-nitrophenylglutaric anhydride gave 46 ± 2% of γ-carbamyl-γ-*p*-nitrophenylbutyric acid and 54 ± 2% of the isomer (Fig. 2).

The figure of ±2 denoting the maximum possible error in the analyses is an estimate based

on two considerations. First, the amic acids were isolated (as a mixture) in >99% yield from the original reaction mixture. Secondly, using known mixtures for chromatography, we found that the titre for the acid in the first band was >98% in each case. (Total recovery of material was >96%.) The composition of the mixture was calculated from the amount of material in the first band. Although the titre did not fall absolutely to zero during the separation of the phenylglutaramic acids (Fig. 1), only 1% of the total acids was eluted between cuts 43 and 47, and cut 45 was chosen as the dividing point. The reproducibility of the analytical figures is shown by the following typical series of results for the percentage of γ-carbamyl-γ-phenylbutyric acid when in admixture with its isomer: (i) 63·3, (ii) 63·7, (iia) 63·3, where (i) and (ii) were obtained in experiments starting from different batches of anhydride and (iia) was the result of a second chromatographic analysis on the same mixture of amic acids as was used in (ii).

The K_a' values, for the isomeric amic acids concerned, corresponded with their relative positions on the chromatograms (Figs. 1 and 2), and it is noteworthy that the greater difference in the K_a' values of the isomers in the case of the *p*-nitrophenylglutaramic acids led to a better separation (Fig. 2).

Anschütz's generalisation, for phenylsuccinic anhydride, does not therefore apply to the α-substituted glutaric anhydrides which we have investigated. Owing to the limited number of reliable results available we consider it inadvisable, at this stage, to advance any generalisations or theoretical considerations concerning the influence of substituent groups on ring scission. However, it is of interest that, in the case of the two α-arylglutaric anhydrides which we have investigated and in which any steric interference may be considered constant, a smaller proportion of the α-amic acid is formed when the α-substituent possesses a greater electron-attracting capacity.

Experimental.

Ethyl (2-Cyanoethyl)phenylmalonate.—Ethyl phenylmalonate (*Org. Synth.*, Coll. Vol. II, p. 288) (1 mol.), in an ethanolic solution containing sodium ethoxide (1 mol.), was condensed with β-chloropropionitrile (prepared in 82% yield from acrylonitrile and dry hydrogen chloride) (1·1 mols.). The product, isolated in the usual way, distilled at 186°/2·5 mm. The oily distillate subsequently crystallised. Recrystallisation from light petroleum (b. p. 40—60°) gave *ethyl (2-cyanoethyl)phenylmalonate* as colourless, irregular prisms, m. p. 41° (32%) (Found: C, 66·9; H, 6·7; N, 4·7. $C_{14}H_{15}O_4N$ requires C, 66·4; H, 6·6; N, 4·8%).

γ-Cyano-α-phenylbutyric Acid.—The foregoing ester (1 mol.) was shaken overnight with a 2·5% solution of potassium hydroxide (3·2 mols.) in dry ethanol. Potassium carbonate was slowly deposited. The mixture was heated under reflux for 2 hours on the steam-bath (in order to complete the hydrolysis) and then cooled and filtered. The filtrate, after neutralisation, concentration (by evaporation of the alcohol), acidification, and extraction with ether, yielded a yellow oil which rapidly solidified. Two crystallisations from ether–light petroleum (b. p. 40—60°) followed by recrystallisation from water gave *γ-cyano-α-phenylbutyric acid* as colourless prisms, m. p. 87° (67%) (Found: C, 70·1; H, 6·0; N, 7·1. $C_{11}H_{11}O_2N$ requires C, 69·8; H, 5·9; N, 7·4%). Decarboxylation had therefore occurred spontaneously after hydrolysis.

γ-Carbamyl-α-phenylbutyric Acid.—A solution of γ-cyano-α-phenylbutyric acid (1 mol.) in concentrated sulphuric acid (6 mols.) was kept for 8 hours at room temperature and was then poured on ice. The semi-solid product was filtered off, washed, and dried (m. p. 58°). Recrystallisation proved difficult. However, preliminary purification (by trituration with ether) followed by slow evaporation of an ethanolic solution of the residue yielded the desired *acid* as square plates, m. p. 60—61° (40%) (Found: C, 63·5; H, 6·5; N, 6·6. $C_{11}H_{13}O_3N$ requires C, 63·8; H, 6·3; N, 6·8%).

Ethyl α-Cyano-α-phenylglutarate.—Prepared by Wideqvist's method (*loc. cit.*), this was a colourless oil, b. p. 187—188°/3 mm. Wideqvist records b. p. 197—198°/8 mm.

γ-Cyano-γ-phenylbutyric Acid.—This acid was obtained by hydrolysis (and simultaneous decarboxylation) of the foregoing ester (1 mol.) with a 10% solution of potassium hydroxide (3·2 mols.) in absolute ethanol and crystallised from carbon tetrachloride as regular octahedra, m. p. 58° (yield, 62%). Wideqvist records m. p. 61°. It had been hoped to devise a more convenient synthesis of this acid by way of ethyl γ-cyano-γ-phenylbutyrate, but two attempts to prepare this ester from benzyl cyanide (1 mol.) and ethyl β-iodopropionate (1·1 mols.), sodamide (1 mol.) being used as condensing agent (cf. Bodroux, *Compt. rend.*, 1910, **151**, 234), were unsuccessful. Dry ether and dry benzene were used as solvents but only a very little product of the correct b. p. was obtained, together with much tarry matter which may have originated from ethyl acrylate produced by the removal of hydrogen iodide from ethyl β-iodopropionate.

γ-Carbamyl-γ-phenylbutyric Acid.—This acid was prepared from the nitrile (as described above for the α-phenyl isomer) and recrystallised from hot water (charcoal) as fine, colourless needles, m. p. 180° (yield, 90%). Wideqvist records m. p. 168° (Found: C, 63·8; H, 6·3; N, 6·5. Calc. for $C_{11}H_{13}O_3N$: C, 63·8; H, 6·3; N, 6·8%).

α-Phenylglutaric Anhydride.—Ethyl (2-carbethoxyethyl)phenylmalonate was obtained (yield, 42·5%) by the usual procedure from ethyl phenylmalonate and ethyl β-iodopropionate and had b. p.

198°/2 mm. (Fichter and Merckens, *Ber.*, 1901, **34**, 4175, give b. p. 219—221°/13 mm.). A trial hydrolysis with 10N-hydrochloric acid, as suggested by these authors, was unsuccessful. Hydrolysis of the ester (1 mol.) was achieved by heating it under reflux with a 10% solution of potassium hydroxide (9·5 mols.) in absolute ethanol. The product was isolated in the usual manner and decarboxylation to α-phenylglutaric acid was completed at 180°. The acid was then heated under reflux with an excess of acetic anhydride. Removal of acetic acid and the excess of acetic anhydride by evaporation *in vacuo* followed by crystallisation of the residue from dry carbon tetrachloride yielded the desired anhydride as colourless rectangular prisms, m. p. 95° (60% calc. on the ester). Fichter and Merckens (*loc. cit.*) record m. p. 95°.

*γ-Cyano-γ-*p*-nitrophenylbutyric Acid.*—(Preliminary experiments established that benzyl cyanide could be converted into *p*-nitrophenylacetamide, m. p. 190°, by nitration of the phenyl nucleus followed by partial hydrolysis of the cyano-group.) γ-Cyano-γ-phenylbutyric acid (17 g.) was dissolved in fuming nitric acid (*d* 1·50; 120 g.) at 10°. The solution was kept at this temperature for 10 minutes and then poured into water, whereupon a small amount of solid separated. The mixture was neutralised with 40% sodium hydroxide solution. Addition of a slight excess over the theoretically required quantity of dilute sulphuric acid liberated the product which was obtained, by extraction with ether and evaporation of the solvent, as a yellow oil. This oil, when scratched under light petroleum (b. p. 100—120°), solidified. Two crystallisations from water and successive recrystallisation from (i) dibutyl ether and (ii) chloroform–carbon tetrachloride yielded *γ-cyano-γ-*p*-nitrophenylbutyric acid* as colourless cubes, m. p. 112° (78%) (Found: C, 56·7; H, 4·4; N, 12·1. $C_{11}H_{10}O_4N_2$ requires C, 56·4; H, 4·3; N, 12·0%). The acid dissolves in 2N-sodium hydroxide solution with production of a blood-red colour. Oxidation of this acid, by boiling it under reflux for ½ hour with an excess of alkaline (sodium carbonate) potassium permanganate solution, yielded *p*-nitrobenzoic acid (m. p. and mixed m. p. with an authentic specimen 238°).

*γ-Carbamyl-γ-*p*-nitrophenylbutyric Acid.*—This acid was prepared from the foregoing γ-cyano-acid in the usual way, and the glutinous product was rendered completely solid by shaking it with ether to remove the soluble impurities. Recrystallisation from water gave an almost theoretical yield of *γ-carbamyl-γ-*p*-nitrophenylbutyric acid* as plates, m. p. 180° (Found: C, 52·9; H, 5·0; N, 11·1%; equiv., by titration, 251. $C_{11}H_{12}O_5N_2$ requires C, 52·4; H, 4·8; N, 11·1%; equiv., 252).

*γ-Cyano-α-*p*-nitrophenylbutyric Acid.*—This *acid* was prepared by nitration of γ-cyano-α-phenylbutyric acid, the same method being used as that described above for γ-cyano-γ-*p*-nitrophenylbutyric acid. The product was obtained by pouring the reaction mixture into water. Recrystallisation from aqueous ethanol gave the acid as colourless, hexagonal plates, m. p. 126° (38%) (Found: C, 56·2; H, 4·0; N, 12·0%). (Extraction with ether of the filtrate from the diluted reaction mixture yielded a further 33% of crude material which could be used directly in the next stage.) The acid of m. p. 126° yielded *p*-nitrobenzoic acid when oxidised with potassium permanganate in alkaline solution.

*γ-Carbamyl-α-*p*-nitrophenylbutyric Acid.*—This *acid* was obtained from the foregoing cyano-acid in the manner already described; it crystallised from water in colourless cubes, m. p. 170° (yield, almost theoretical) (Found: C, 52·7; H, 5·0; N, 11·0%; equiv., 252).

*α-(*p*-Nitrophenyl)glutaric Anhydride.*—Several methods for preparing α-*p*-nitrophenylglutaric acid were investigated, *viz.*, (*a*) from α-phenylglutaric acid by nitration; (*b*) from γ-carbamyl-γ-*p*-nitrophenylbutyric acid by three routes: (i) by alkaline hydrolysis; (ii) by treatment with nitrous acid (cf. Bouveault, *Bull. Soc. chim.*, 1892, **9**, 368; Sudborough, *J.*, 1895, **67**, 602); (iii) by acid hydrolysis. Of these methods only (*b*) (iii) gave a reasonable yield of the desired product. The amic acid (3·75 g.) was heated under reflux for 15 minutes with 70% (w/w) sulphuric acid (10 ml.). When the hydrolysate was poured into water an almost black, amorphous solid was obtained. A solution of this material in aqueous sodium carbonate was boiled with decolorising charcoal and filtered. The filtrate, on acidification and cooling, gave the desired acid (2·2 g., 59%) as a light yellow, ether-soluble solid, m. p. 138°. *α-*p*-Nitrophenylglutaric anhydride*, prepared from the acid by treatment with acetic anhydride in the usual way, was insoluble in cold chloroform, carbon tetrachloride, and light petroleum, sparingly soluble in ether, and moderately soluble in benzene and dioxan. Crystallisation from dry light petroleum (b. p. 60—80°) (charcoal) or from dry benzene yielded the anhydride as pale yellow needles, m. p. 136° (42%) (Found: C, 56·2; H, 3·9; N, 5·8. $C_{11}H_9O_5N$ requires C, 56·2; H, 3·9; N, 6·0%). The anhydride was insoluble in cold 10% sodium hydrogen carbonate solution and was only very slowly soluble in cold 2N-sodium hydroxide solution, with which it gave a yellow colour.

*γ-*p*-Aminophenyl-γ-carbamylbutyric Acid.*—After a trial hydrogenation of *p*-nitrophenylacetic acid with subsequent benzoylation to *p*-benzamidophenylacetic acid (m. p. 205°), γ-carbamyl-γ-*p*-nitrophenylbutyric acid (2·5 g.) was hydrogenated in ethanolic solution at room temperature and slightly more than atmospheric pressure, Raney nickel being used as catalyst. The uptake of hydrogen was almost theoretical for one nitro-group. A little water was added and the mixture was boiled and filtered. Evaporation *in vacuo* of the filtrate to dryness yielded the amino-acid (2 g., 91%) as a yellow amorphous solid. (The presence of the amino-group was confirmed by the usual test.)

*γ-*p*-Benzamidophenyl-γ-carbamylbutyric Acid.*—The amino-acid (1·5 g.) was benzoylated by shaking it for 15 minutes with benzoyl chloride (1·5 ml.) in an excess of 10% sodium hydrogen carbonate solution. The reaction mixture was acidified and the product was freed from benzoic acid by extraction of the latter with ether. Repeated recrystallisation from aqueous ethanol yielded *γ-*p*-benzamidophenyl-γ-carbamylbutyric acid* as colourless needles, m. p. 234° (41%) (Found: C, 66·0; H, 5·4; N, 8·7%; equiv., 328. $C_{18}H_{18}O_4N_2$ requires C, 66·3; H, 5·6; N, 8·6%; equiv., 326). This amic acid was insoluble in chloroform and butanol; it was sparingly soluble in dioxan and in β-ethoxyethanol.

*α-*p*-Benzamidophenyl-γ-carbamylbutyric Acid.*—This *acid* was prepared in a similar manner from γ-carbamyl-α-*p*-nitrophenylbutyric acid and recrystallised from very dilute alcohol as rosettes of needles, m. p. 207° (yield, 35%) (Found: C, 66·1; H, 6·0; N, 8·6%). The solubility properties of this amic acid were similar to those of its isomer.

α-p-*Benzamidophenylglutaric Acid and Anhydride.*—(i) *From γ-p-benzamidophenyl-γ-carbamylbutyric acid.* The acid was boiled under reflux with 70% sulphuric acid for 30 minutes and the benzoic acid produced was removed by steam-distillation. The residual solution was neutralised with sodium hydroxide solution, and the α-*p*-aminophenylglutaric acid (in solution as its disodium salt) was then benzoylated and isolated in the usual way. The benzoylated amino-acid, after crystallisation from aqueous ethanol, was obtained (yield, 56%) as rosettes of needles, m. p. 194°. Treatment of this acid with acetic anhydride and crystallisation of the product from acetic anhydride–benzene gave α-p-*benzamidophenylglutaric anhydride* as minute, colourless cubes, m. p. 208° (yield, 85%) (Found: C, 69·4; H, 4·6; N, 4·9. $C_{18}H_{15}O_4N$ requires C, 69·9; H, 4·9; N, 4·5%). It was insoluble in 10% sodium hydrogen carbonate solution.

(ii) *From* α-p-*nitrophenylglutaric acid.* The acid (10 g.), dissolved in ethanol (150 ml.), was hydrogenated at 100 atm. pressure by aid of Raney nickel as catalyst. The amino-acid was isolated, benzoylated, and converted into the anhydride in the usual ways. The product (yield, 41·5%) was identical with that obtained by method (i).

Determination of K_a' *Values of the Substituted Glutaramic Acids.*—Values of K_a' for four of the acids (see Figs. 1 and 2) were determined by electrometric titration, a glass electrode and a Cambridge pH Meter being used.

Preparation of the Solution for Chromatographic Analysis.—A stream of dry ammonia was passed for 30 minutes into a solution of the anhydride (500 mg.) in dry dioxan (50 ml.). The reaction vessel was contained in a thermostat (20°) and was shaken intermittently. A little water was used to remove some of the product which adhered to the thermometer and inlet tube, and the aqueous dioxan was distilled *in vacuo*. To the dried ammonium salts was added an exact equivalent of dilute sulphuric acid solution required to liberate the free amic acids which were obtained in the dry state, together with ammonium sulphate, by evaporation *in vacuo*. The residue was warmed with dry acetone, and the remaining ammonium sulphate filtered off and washed with more acetone. The combined filtrate and washings were made up to 100 ml. with the same solvent. Titration of a portion of this solution with 0·01N-alkali showed, in each case, that not more than 1% of material had been lost by this stage. Moreover, the acetone solution was shown to be free from sulphate ion. A measured volume of the acetone solution (containing *ca.* 100 mg. of material) was evaporated to dryness *in vacuo* and the residue was analysed chromatographically.

Chromatographic Procedure.—Separations of the isomeric amic acids were achieved, in each case, by employing a column of kieselguhr with an immobile phase of 2M-phosphate buffer solution and a mobile phase of *n*-butanol–chloroform. The buffer solution, the optimum pH for which had been determined by previous experiments as 7·76, within narrow limits, was mixed with purified "Supercel" kieselguhr to form a gel containing 50% (w/w) of the aqueous solution. Approx. 30 g. of this material were made into a slurry in the appropriate mobile phase and introduced into a Pyrex tube, 30 cm. long and 1·5 cm. in diameter. Packing was facilitated by means of a perforated brass disc, fitted with a long handle, which was used first to agitate the gel to remove entrapped air and then to pack the material by slowly tamping down successive layers to give a column of length 20 cm. The upper surface of the gel was protected with a thin layer of glass wool and the column was then washed with 200 ml. of the mobile phase, previously equilibrated with the appropriate buffer solution. The sample for analysis (*ca.* 100 mg.) was taken up in the minimum amount of the mobile phase and the solution was loaded on to the top of the column in the normal manner. The chromatogram was developed with *n*-butanol–chloroform mixtures under positive pressure; the apparatus was designed to enable the solvent reservoir to be refilled without releasing the pressure on the top of the column. An automatic fraction-cutting machine of the gravity type (Randall and Martin, *Biochem. J.*, 1949, **44**, Proc., ii) collected cuts of 5·8 ml. each, which were titrated with 0·01N-sodium hydroxide to cresol-red (Moyle, Baldwin, and Scarisbrick, *loc. cit.*). A graph was constructed of titre, after correction for a blank value, against cut number. This enabled the amount of material in each peak to be determined and hence the proportion of the two amic acids in the original solution to be calculated (Figs. 1 and 2).

Isolation of the Amic Acids after Chromatography.—The titrated cuts corresponding to one peak were combined, and dilute sulphuric acid, exactly equivalent to the total titre of sodium hydroxide, was added. The solution was evaporated to dryness *in vacuo* and the product dried *in vacuo* over phosphoric oxide. The amic acid was then obtained by extraction with hot dry acetone, and filtration and evaporation of the acetone solution, followed by recrystallisation of the residue from a suitable solvent.

Identification of the Products after Chromatography.—The structures of the two phenylglutaramic acids and of the two *p*-nitrophenylglutaramic acids were established by comparison (m. p. and mixed m. p.) with their respective samples which had been synthesised by unequivocal routes. Identity was proved in all four cases. It is noteworthy that the γ-carbamyl-γ-phenylbutyric acid, isolated by chromatographic analysis, had m. p. 180° alone or after admixture with the material synthesised by Wideqvist's method. The authenticity of our compound and the correctness of its m. p. were thus established.

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THE UNIVERSITY, NOTTINGHAM. [*Received, March 17th*, 1951.]

519. *The Influence of the Nitro-group on the Reactivity of Aromatic Halogens. Part I.*

By C. W. L. Bevan.

Arrhenius parameters have been determined for the bimolecular substitution reaction of the *p*-halogenonitrobenzenes with sodium ethoxide in absolute ethyl alcohol, a reaction free from steric effects. A possible explanation of the order of reactivity is suggested.

The comparative rates of replacement of halogen atoms attached to a saturated carbon atom have been determined in a number of instances (*e.g.*, Polanyi *et al.*, *Trans. Faraday Soc.*, 1936, **32**, 843; Cooper and Hughes, *J.*, 1937, 1183; Miller and Bernstein, *J. Amer. Chem. Soc.*, 1948, **70**, 3600). In all cases the sequence of reactivity is RI > RBr > RCl > RF and the spread of reactivity of the order of 10^5—10^6. For the reactions of halogenobenzenes with sodium methoxide, and with pyridine (Tronow and Kruger, *J. Russ. Phys. Chem. Soc.*, 1926, **58**, 1270; cf. *Chem. Zent.*, 1927, **2**, 1145), the rate sequence is PhI > PhBr > PhCl > PhF with a spread of reactivity less than 10^2 (cf. Bergstrom *et al.*, *J. Org. Chem.*, 1936, **1**, 170, 179). The presence of electron-attracting substituents in the halogenobenzenes and in halogenated heterocyclic molecules causes a reversal of this order of reactivity. For instance, the reactions of monohalogeno-2 : 4-dinitrobenzenes with ethoxide ions gave the relative rates: RCl, 3·26; RBr, 1·89; RI, 0·55 (Lulofs, *Rec. Trav. chim.*, 1901, **20**, 292). These reactions were found to be retarded by the addition of water and were established as bimolecular (cf. Hughes, *Trans. Faraday Soc.*, 1941, **37**, 627). Further, Holleman (*Rec. Trav. chim.*, 1904, **23**, 256) found that with methoxide ions 2 : 4-dinitrofluorobenzene reacts some 600 times as fast as the corresponding chlorobenzene (cf. Miller and Wrightson, Amer. Chem. Soc. Meeting, Sept. 17th, 1947, Abstr. of Papers, p. 16); Chapman *et al.*, *Chem. and Ind.*, 1951, **8**, 148).

The results of the present investigation, which are free from possible complications due to *ortho*-substituents, are shown in Table I.

TABLE I.

k_2 (*in l. mol.*$^{-1}$ *sec.*$^{-1}$) *for* p-*halogenonitrobenzenes with* OEt^- *in absolute* EtOH.

p-Substituent:	F.		Cl.		Br.		I.	
Temp.	k_2.	Temp.	k_2.	Temp.	k_2.	Temp.	k_2.
90·80°	$2·20 \times 10^{-1}$	90·80°	$9·63 \times 10^{-4}$	90·80°	$8·38 \times 10^{-4}$	90·80°	$7·09 \times 10^{-5}$
80·80	$1·04 \times 10^{-1}$	100·7	$2·00 \times 10^{-3}$	101·0	$1·80 \times 10^{-3}$	—	—
70·00	$4·73 \times 10^{-1}$	109·8	$3·82 \times 10^{-3}$	111·1	$3·73 \times 10^{-3}$	—	—
60·80	$2·08 \times 10^{-3}$	120·0	$7·60 \times 10^{-3}$	120·1	$6·87 \times 10^{-3}$	—	—
50·80	$8·56 \times 10^{-3}$	129·8	$1·42 \times 10^{-2}$	130·1	$1·31 \times 10^{-2}$	—	—

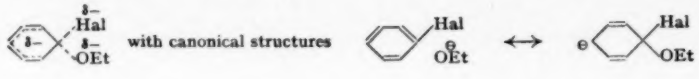
Arrhenius parameters.

p-Substituent	F	Cl	Br
$\log_{10} B$ (l. mol.$^{-1}$ sec.$^{-1}$)	10·7	9·0	9·2
E (kcals.)	19·0	20·1	20·3

Arrhenius parameters were not determined for the iodide since the rate constants decreased rapidly after the first 10% of reaction and iodine was liberated, owing possibly to incursion of a free-radical reaction. The rate quoted is calculated from velocity constants determined over the first 10% of reaction.

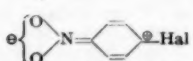
These results show that the inverted sequence of reactivity persists even in the absence of *ortho*-substituents, and that the large difference in rate between the fluoro- and the chloro-compound is reflected in both parameters of the Arrhenius equation.

The spread of reactivity is of the order 10^2—10^4 which is considerably less than in saturated halides. Formation of the transition state of aliphatic substitution, *viz.*, $\overset{\delta-}{\text{Hal}} \cdots \text{R} \cdots \overset{\delta-}{\text{OEt}}$, requires a stretching of the carbon–halogen bond in order partly to accommodate the electron pair of the attacking entity. In aromatic substitution (cf. Hughes and Ingold, *J.*, 1941, 608) the transition state may be pictured as

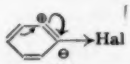


Here the electron pair of the attacking entity may resonate in part through the unsaturated system, so enabling the transition state to be formed without much stretching of the C–Hal bond. Changing the halogen should thus have a smaller effect on the reaction rate than in the aliphatic case.

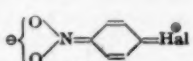
The sequence of halogen reactivity in aliphatic systems is probably largely determined by bond strengths, and the inductomeric polarisability of the halogens which is believed to be in the order I > Br > Cl > F (cf. Baker and Hopkins, *J.*, 1949, 1089). However, in the aromatic case, the energetics of bond formation is likely to be the rate-determining factor. Exchange reactions of the phenyl halides are facilitated by electron-attracting substituents, and it has been pointed out by Catchpole, Hughes, and Ingold (*J.*, 1948, 10) that this must be correlated with the resulting reduction in π-electron screening of the reactive carbon atom, which is thus rendered more vulnerable to nucleophilic attack. The effects of the halogens may be considered on the following basis: in unsubstituted benzene there is one π-electron associated with each carbon atom, whereas in *p*-nitrohalogenobenzenes the nitro-group will tend, both by its tautomeric and by its inductive attraction, to reduce this to some fraction of unity by causing contributions from structures such as (*A*). The inductive effect of the halogen atoms, acting mainly on the electrons of the carbon–halogen bond, will increase the electronegativity of the reactive carbon atom and produce, in the π-electrons of the ring, displacements as in (*B*). This will tend to lower slightly the reduction in π-electron screening caused by the nitro- or other electron-attracting group. Moreover, mesomeric release by the halogen atom will produce a fractional π-electron at the reactive carbon atom by contributions from structures such as (*C*).



(*A.*)



(*B.*)



(*C.*)

Thus, both by its inductive and by its mesomeric effects the halogen atom will tend to oppose the reduction in π-electron screening caused by the nitro-group. The order of inductive attraction for the halogens is F > Cl > Br > I, so that, if the sequence of reactivity of the nitrohalogenobenzenes in the type of reaction under discussion, *viz.*, F > Cl > Br > I, is determined by the order in which they reduce the π-electron screening at the reactive carbon atom in the ground states of these molecules, then the order of mesomeric release leading to structures such as (*C*) would be I > Br > Cl > F. Such a sequence has been postulated by Evans and Walker (*Trans. Faraday Soc.*, 1944, **40**, 384), and Hurdis and Smythe (*J. Amer. Chem. Soc.*, 1942, **64**, 2212) have suggested from their measurements of the dipole moments of the *p*-nitrohalogenobenzenes that the nitro-group effects a greater increase in the double bond character of the C–Cl bond than of the C–F bond.

There is, however, a considerable weight of evidence summarised by Baker and Hopkins (*loc. cit.*) in favour of a mesomeric release by the halogens following the reverse order, and it must be stated that the combined influence of the effects discussed above is of too intricate a character to enable one to be emphatic regarding the conclusion of the analysis, which must, therefore, be regarded as tentative.

Experimental.

Preparation of Materials.—The *p*-chloro-, *p*-bromo-, and *p*-iodo-nitrobenzenes were commercial specimens recrystallised to constant m. p. from ligroin, giving respectively, m. p. 83·1°, 127·0°, 174·0°. *p*-Fluoronitrobenzene was prepared by Schiemann and Pillarsky's method (*Ber.*, 1929, **62**, 6040), b. p. 80·5°/9·5 mm., m. p. 27·5°.

Commercial absolute alcohol was dried by Smith's method (*J.*, 1927, 1288) as modified by Manske (*J. Amer. Chem. Soc.*, 1931, **53**, 1106) and fractionated. It was stored under oxygen-free nitrogen and delivered for use under pressure.

Kinetic Measurements.—Thermostat temperatures below 100° were steady to within ±0·01°; those above 100° to within ±0·05°. The method of sealed tubes was used at all temperatures. Aliquots of approx. 0·02M-solutions of the halides and of an approx. 0·04M-solution of sodium ethoxide in absolute ethyl alcohol were mixed (at −80° in the case of the fluoride), sealed, and immersed in a thermostat for definite periods of time. Zero readings were obtained from tubes immersed for 5 minutes. The reaction was stopped by rapidly cooling and then opening the tubes under a few ml. of 2N-nitric acid; the halide ion produced in reaction was determined by titration with 0·02M-silver nitrate solution, a valve potentiometer being used. In the case of the fluoride, the tubes were opened under an aliquot of standard acid, and excess of acid determined by back-titration with standard carbon-dioxide-free alkali, with bromothymol-blue as indicator.

Rate constants were calculated from the formula :

$$k_2 = \frac{1}{(a-b)t} \log_e \left[\frac{b(a-x)}{a(b-x)}\right]$$

where a and b are the initial concentrations of sodium ethoxide and alkyl halide respectively, and x is the decrease in concentration after time t. The results of typical experiments are shown in detail in Table II. A correction for solvent expansion between the temperature at which the tubes were filled and that at which the rate was measured was applied. Rate constants could be duplicated to within $\pm 1\%$.

TABLE II.

Determination of rate constants, k_2, in sec.$^{-1}$ g.-mol.$^{-1}$. Reaction of p-fluoronitrobenzene with sodium ethoxide in absolute ethyl alcohol.

Initially, [Halide] ~ 0·01M. and [NaOEt] ~ 0·02M.

Concns. are expressed in ml. of 0·01190N-NaOH per 9·65 ml. sample. Temp. = 80·80°.

t (min.)	0	4	5	6	7	8	10	13	16	20	25
[NaOEt]	14·14	12·09	11·72	11·43	11·18	10·79	10·41	9·77	9·41	8·89	8·58
[Halide]	6·60	4·55	4·18	3·89	3·64	3·25	2·87	2·23	1·87	1·35	1·01
10^2k_2	—	9·64	9·64	9·45	9·22	9·81	9·44	9·87	9·56	10·10	9·67

Mean $k_2 = 9·63 \times 10^{-2}$; corrected for solvent expansion (16—81°), $k_2 = 1·04 \times 10^{-1}$.

Reaction of p-chloronitrobenzene with sodium ethoxide in absolute ethyl alcohol.

Initially [Halide] ~ 0·01M. and [NaOEt] ~ 0·02M.

Concns. are expressed in ml. of 0·0200N-$AgNO_3$ per 9·65 ml. sample. Temp. = 129·8°.

t (min.)	0	5	10	15	25	35	40	65	70	116	145
[NaOEt]	10·20	9·87	9·57	9·30	8·77	8·34	8·20	7·61	7·48	6·64	6·48
[Halide]	4·62	4·29	3·99	3·72	3·19	2·76	2·62	2·03	1·90	1·06	0·90
10^2k_2	—	1·18	1·19	1·20	1·24	1·29	1·26	1·17	1·19	1·30	1·18

Mean $k_2 = 1·22 \times 10^{-2}$; corrected for solvent expansion (18—130°), $k_2 = 1·42 \times 10^{-2}$.

I am much indebted to Professor E. D. Hughes, F.R.S., for discussions and helpful criticisms. The investigation was commenced at the William Ramsay and Ralph Forster Laboratories, University College, London.

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520. *The Chemotherapy of Tuberculosis. Part III.* Some N-Substituted 4-Aminodiphenyls.*

By L. BAUER, J. CYMERMAN, and W. J. SHELDON.

To investigate the effect of basic strength on antituberculous activity, two *N*-alkyl derivatives of 4-aminodiphenyl, *viz.*, *N'*-4'-diphenylyl-*NN*-diethylethylenediamine, and *N*-4'-diphenylyl-2-morpholinoethylamine, have been prepared, as well as the oxygen analogue of the former, 4-2'-diethylaminoethoxydiphenyl.

The highly basic 4-diphenylyl-guanidine and -diguanide were obtained, and the latter was found to condense readily with acetone to give a dihydrotriazine.

Condensation of 4-aminodiphenyl with a number of substituted benzaldehydes gave the expected Schiff's bases which were reduced catalytically to the 4-benzylaminodiphenyls.

THE importance of primary aromatic amines in the chemotherapy of tuberculosis was early appreciated (Kuroya, *Jap. J. Exp. Med.*, **1929**, **7**, 255). Systematic investigation of a series of these bases by Erlenmeyer *et al.* (*Helv. Chim. Acta*, **1945**, **28**, 1406; **1947**, **30**, 539, 2058; **1948**, **31**, 75, 991; **1949**, **32**, 605, 1209, 1275, 1674) and by Doub and Youmans (*Amer. Rev. Tuberc.*, **1950**, **61**, 407) showed the most active compounds to be 4-aminodiphenyl, its isosteres, and β-naphthylamine.

Antibacterial activity in basic compounds has been found to depend on a high degree of ionisation (greater than 50%) at the physiological pH (7·2) (Albert, Rubbo, *et al.*, *Brit. J. Exp.*

* Part II, *J.*, 1950, 2078.

Path., 1945, **26**, 160; 1949, **30**, 159). In the cases of both 4-aminodiphenyl (pK_a 4·27 at 25°; Hall and Sprinkle, *J. Amer. Chem. Soc.*, 1932, **54**, 3474) and β-naphthylamine (pK_a 4·3 at 25°; Farmer and Warth, *J.*, 1904, 1726) the percentage of base ionised at pH 7·2 does not exceed 1%; the antibacterial properties of these compounds might therefore conceivably be improved by an increase in their ionisation, provided of course that their antituberculous activity resides in the cations and not in the undissociated molecules.

The aim of the present work was then to discover the correlation between basicity and antibacterial activity in a series of *N*-substituted derivatives of 4-aminodiphenyl and β-naphthylamine, and concurrently to examine two other effects: (*a*) variation in lipoid solubility, and (*b*) possession of optimal flat surface area, in view of the results of Albert *et al.* (*loc. cit.*) showing that, even in highly ionised compounds, possession of a minimal flat area was essential for antibacterial activity to appear. It should be noted that both diphenyl and naphthalene exist as planar molecules (Dhar, *Indian J. Physics*, 1932, **7**, 43; Robertson, *Proc. Roy. Soc.*, 1933, *A*, 142, 674).

In the *N*-4-diphenylylamidines previously reported (Part I, *J.*, 1950, 1826), basicity has been increased to the required extent (pK_a 8—9 at 20°; forthcoming publication) and at the same time an isomeric series of *p*-phenylbenzamidines was prepared (*J.*, 1950, 2078) which was devoid of the *p*-aminobenzoic acid skeleton claimed to be essential for activity. After this work had been completed, the papers by Partridge *et al.* (*J.*, 1949, 3043; 1950, 459) came to hand, in which a series of *p*-alkoxy- and *p*-ω-alkoxyalkoxy-*N*-arylbenzamidines are described and examined for antituberculous activity, and we now learn that *N*-4-diphenylylbenzamidine, described in Part I of our series, was prepared independently in Dr. Short's laboratory in 1948 (Drs. D. A. Peak and W. F. Short, private communication). We wish to express our apology to Drs. Peak and Short and their collaborators for the lack of consultation in this matter. An extensive series of publications, concerned with direct correlation between chemical structure and antituberculous activity, is now emanating from Dr. Short's laboratory (*J.*, 1949, 2680, 2683, 3043; 1950, 445; and subsequent papers).

The present paper describes the preparation of some *N*-alkyl derivatives of 4-aminodiphenyl, prepared in order to obtain compounds of basic strength intermediate between those of the primary amine and of the amidines described earlier. Condensation of 4-aminodiphenyl and 2-diethylaminoethyl chloride afforded *N'*-4'-diphenylyl-*NN*-diethylethylenediamine (I) which on treatment with methyl iodide gave the dimethiodide of *N'*-4'-diphenylyl-*NN*-diethyl-*N'*-methylethylenediamine. Reaction of 4-aminodiphenyl with 2-morpholinoethyl chloride gave the analogous *N*-4'-diphenylyl-2-morpholinoethylamine (II), which with methyl iodide similarly afforded the dimethiodide of *N*-4'-diphenylyl-*N*-methyl-2-morpholinoethylamine. Treatment of 4-hydroxydiphenyl with 2-diethylaminoethyl chloride gave the oxygen analogue, 4-2'-diethylaminoethoxydiphenyl (III).

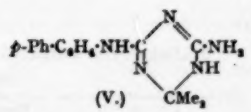
It is well known that the introduction of a guanidine or diguanide grouping leads to an even greater enhancement of basic strength; thus phenylguanidine and *N'*-phenyldiguanide have pK_a 10·9 (Davies and Elderfield, *J. Amer. Chem. Soc.*, 1932, **54**, 1499) and 10·7 (Albert *et al.*, *loc. cit.*) respectively at 20°; moreover the guanidinium and diguanide ions are themselves flat (cf. Tutida, *Rev. Phys. Chem. Jap.*, 1939, **13**, 31; Albert *et al.*, *loc. cit.*). Reaction of 4-aminodiphenyl with *S*-methylthiuronium sulphate at 200° afforded 60% of 4-diphenylylguanidine, and condensation of 4-diphenylylammonium benzenesulphonate and dicyandiamide at 155—180° gave *N'*-4-diphenylyldiguanide (IV). The free diguanide on prolonged boiling with acetone afforded a compound which is believed to be 6-amino-4-4'-diphenylylamino-1 : 2-dihydro-2 : 2-dimethyl-1 : 3 : 5-triazine (V) for which it gave correct analytical figures.

p-Ph·C$_6$H$_4$·NH·CH$_2$·CH$_2$·NEt$_2$ (I.)

p-Ph·C$_6$H$_4$·NH·CH$_2$·CH$_2$·N<[(CH$_2$)]$_4$>O (II.)

p-Ph·C$_6$H$_4$·O·CH$_2$·CH$_2$·NEt$_2$ (III.)

p-Ph·C$_6$H$_4$·NH·C(:NH)·NH·C(:NH)·NH$_2$ (IV.)



(V.)

A similar easy formation of a dihydrotriazine by condensation of a diguanide with acetone has been reported by Birtwell, Curd, Hendry, and Rose (*J.*, 1948, 1650). Treatment of this base with benzenesulphonic acid afforded two salts; one analysed correctly for the expected triazine dibenzenesulphonate, and the other for *N*-4-diphenylylguanidinium benzenesulphonate, with

which it was identical (mixed m. p.). No previous instance has, to the authors' knowledge, been recorded of such a ready degradation of a dihydrotriazine to a guanidine.

The preparation of Schiff's bases from tuberculostatically active aromatic amines was reported by Erlenmeyer (*Helv. Chim. Acta*, 1945, **28**, 1413). Condensation of 4-aminodiphenyl with *p*-hydroxy-, *p*-dimethylamino-, *p*-nitro-, and *p*-methoxy-benzaldehyde gave the expected benzylidene derivatives in quantitative yield. Reduction of the methine linkage by means of sodium and alcohol (Fischer, *Annalen*, 1887, **241**, 330) having proved unsuccessful for these compounds, uptake of hydrogen (1 mol.) in the presence of Adams's catalyst proceeded smoothly at atmospheric pressure to give the required benzylamino-compounds; 4-benzylaminodiphenyl was prepared similarly.

These bases and the corresponding hydrochlorides showed the expected properties, with the exception of the *N*-*p*-hydroxybenzyl compound which possessed in abnormally high m. p. (288°) and resisted all attempts to form a hydrochloride. It is therefore concluded that this compound exists predominantly in the zwitterionic form owing to internal salt formation. Catalytic reduction of 4-*p*-nitrobenzylideneaminodiphenyl did not proceed normally, but gave a mixture of products (cf. Phillips and Maggiolo, *J. Org. Chem.*, 1950, **15**, 659) which were not further investigated.

Experimental.

N'-4'-Diphenylyl-NN-diethylethylenediamine.—4-Aminodiphenyl (8·5 g.) was refluxed for 6 hours with a solution of 2-diethylaminoethyl chloride hydrochloride (10 g.) in aqueous alcohol (100 c.c.; 70%) containing potassium carbonate (10 g.). Water was added to the cooled mixture and the oil which separated was taken up in ether and dried (KOH). Distillation gave the base (9·7 g., 71%) as a light yellow viscous oil, b. p. 232—244°/11 mm., n_D^{15} 1·6050. The *dibenzenesulphonate* crystallised from methanol–ether in white needles, m. p. 124·5° (Found: C, 61·7; H, 6·3; N, 4·2, 5·3, 5·3. $C_{30}H_{32}O_6N_2S_2$ requires C, 61·6; H, 6·2; N, 4·8%). This salt was very soluble in water. The very hygroscopic *dihydrochloride* formed clusters of needles, m. p. 138° (sealed tube), from methanol–ether (Found: C, 8·0. $C_{18}H_{26}N_2Cl_2$ requires N, 8·2%). The *picrate* crystallised in yellow needles, m. p. 138—139° (Found: C, 57·5; H, 5·3; N, 14·3, 14·3. $C_{34}H_{32}O_{14}N_8$ requires C, 57·9; H, 5·5; N, 14·1%).

Treatment of a solution of the base in ether with an excess of methyl iodide at room temperature gave an oil which after several months solidified and then afforded colourless rhombic plates (from ethanol), m. p. 148° (Found: C, 43·9; H, 5·7; N, 4·6. $C_{21}H_{32}N_2I_2$ requires C, 44·5; H, 5·7; N, 4·9), and was *N'-4'-diphenylyl-NN-diethyl-NN'N'-trimethylethylenediammonium di-iodide.*

N-4'-Diphenylyl-2-morpholinoethylamine.—4-Aminodiphenyl (8·5 g.) and 2-morpholinoethyl chloride hydrochloride (19 g.; Mason and Black, *J. Amer. Chem. Soc.*, 1940, **62**, 1443) similarly (5 hours) afforded an oil which, dried (K_2CO_3) in ether, gave the *base* (9 g., 64%) as a viscous yellow oil, b. p. 210—230°/0·02 mm., which slowly solidified and crystallised from light petroleum (b. p. 40—70°) in needles, m. p. 80—81° (Found: N, 9·95. $C_{18}H_{22}ON_2$ requires N, 9·95%). Treatment with dry alcoholic hydrogen chloride and then ether gave the *dihydrochloride*, m. p. 240° (decomp.) with previous softening at 180° (Found: N, 7·4. $C_{18}H_{24}ON_2Cl_2$ requires N, 7·9%). Treatment of the base with a solution of methyl iodide in ether gave, on storage, a gum which solidified when boiled with ethyl acetate to yield the *dimethiodide* as white micro-crystals, m. p. 240° (decomp.) (Found: C, 43·5; H, 5·75. $C_{21}H_{30}ON_2I_2$ requires C, 43·45; H, 5·2%).

Table I.

p'-*Substituted* 4-*Benzylideneaminodiphenyls*, R·C_6H_4·CH:N·C_6H_4Ph.

			N, %.	
R.	M. p.	Formula.	Found.	Reqd.
NH_2 *	152—153°	$C_{19}H_{16}N$	5·75	5·45
OH	249—250	$C_{19}H_{15}ON$	5·1	5·1
NMe_2	210—211	$C_{21}H_{20}N_2$	9·8	9·3
OMe	163—164	$C_{20}H_{17}NO$	5·35	4·9
NO_2	179—180	$C_{19}H_{14}N_2O_2$	9·35	9·25

* Previously prepared by Bell and Kenyon, *J.*, 1926, 2705, who give m. p. 148°.

Table II.

p'-*Substituted* 4-*Benzylaminodiphenyls*, R·C_6H_4·CH_2·NH·C_6H_4Ph.

	Base.								Hydrochloride.		
			Found, %.			Reqd., %.				Found,	Reqd.,
R.	M. p.	Formula.	C.	H.	N.	C.	H.	N.	M. p.	N, %.	N, %.
H	100—101°	$C_{19}H_{17}N$	87·9	6·55	5·5	88·0	6·6	5·4	200—201°	4·7	4·75
OH	288	$C_{19}H_{17}NO$	—	—	5·45	—	—	5·1	—	—	—
NMe_2	113·5—114	$C_{21}H_{22}N_2$	—	—	9·3	—	—	9·25	265	7·8	7·5
OMe	118—120	$C_{20}H_{19}NO$	87·5	7·05	—	87·85	7·05	—	272—273	4·6	4·3

4-2′-*Diethylaminoethoxydiphenyl*.—4-Hydroxydiphenyl (5·4 g.) was added to a solution of sodium methoxide [from sodium (1·5 g.)] in methanol (100 c.c.), followed by 2-diethylaminoethyl chloride hydrochloride (6 g.), and the mixture was refluxed for 4 hours. Excess of methanol was removed *in vacuo* and the residue distilled to give a mixture of the phenol and the required basic ether (b. p. 135—145°/0·06 mm.). Trituration with light petroleum (b. p. 40—70°) left the unchanged phenol which was filtered off, and evaporation of the solvent afforded the base as an oil, n_D^{17} 1·5746, which readily gave a *hydrochloride* crystallising from ethanol–ether in silky needles, m. p. 179—179·5° (Found: N, 4·7. $C_{18}H_{24}ONCl$ requires N, 4·6%). The *methiodide* (prepared in ether), m. p. 194—194·5°, separated from methanol–ether in rhombs (Found: N, 3·3. $C_{19}H_{26}ONI$ requires N, 3·4%). Both the hydrochloride and the methiodide were very soluble in water. The *picrate* formed golden-yellow plates (from alcohol), m. p. 91—92° (Found: N, 11·1. $C_{24}H_{27}O_8N_4$ requires N, 11·3%). The preparation of this base is claimed in B.P. 521,575 (*Chem. Abs.*, 1942, **36**, 783) where b. p. 154—157°/1·1 mm. is given.

4-*Diphenylylguanidine*.—4-Aminodiphenyl (8 g.) and *S*-methylthiuronium sulphate (5·5 g.) was heated at 200° for 2·5 hours, whereafter evolution of methanethiol had ceased. The cooled mass was triturated with ether and then cold water, and then boiled with water (300 c.c.) and filtered. The undissolved residue (6 g., 60%) was insoluble in ether or acetone, and crystallised from methanol–ether as white needles, m. p. 245°, of 4-*diphenylylguanidine sulphate* (Found: N, 16·15, 16·2. $C_{26}H_{28}O_4N_6S$ requires N, 16·15%). A solution of this salt in N-hydrochloric acid was treated with hot aqueous barium hydroxide until neutral (Congo-red), the precipitated barium sulphate filtered off, and the filtrate basified at 0° with barium hydroxide solution. The precipitate (85% yield) crystallised from chloroform–light petroleum in rhombic plates of 4-*diphenylylguanidine*, m. p. 189—190° (Found: C, 73·5; H, 6·4. $C_{13}H_{13}N_3$ requires C, 73·9; H, 6·15%). The *picrate* separated from dilute methanol as yellow prisms, m. p. 253° (decomp.) (Found: N, 19·15. $C_{19}H_{16}O_7N_6$ requires N, 19·1%) and the *benzenesulphonate* from *iso*propyl alcohol as nacreous plates, m. p. 170—171° (Found: N, 11·2. $C_{19}H_{19}O_3N_3S$ requires N, 11·4%).

N′-4-*Diphenylyldiguanide*.—A mixture of 4-diphenylylammonium benzenesulphonate (8·35 g.) and dicyandiamide (2·2 g., 1·04 mols.) was heated at 155° (bath-temp.) for 1·5 hours and then at 180° for a further 1·5 hours. The cooled solid was boiled with water (500 c.c.) and filtered, the filtrate on cooling affording white needles (3·1 g., 30%) of N′-4-*diphenylyldiguanide benzenesulphonate*, m. p. 214° (Found: N, 17·45. $C_{20}H_{21}O_3N_5S$ requires N, 17·05%). The aqueous filtrate from this salt was basified and the precipitated base crystallised, after prolonged boiling, from dilute acetone to give white crystals (1·5 g., 21%), m. p. 181—182° (decomp.), separating from light petroleum–chloroform as prisms of unchanged m. p., of 6-*amino*-4-4′-*diphenylylamino*-1 : 2-*dihydro*-2 : 2-*dimethyl*-1 : 3 : 5-*triazine* (Found: C, 69·6; H, 6·35; N, 23·6, 23·3. $C_{17}H_{19}N_5$ requires C, 69·7; H, 6·5; N, 23·8%). This base on treatment with a solution of benzenesulphonic acid in methanol afforded prisms, m. p. 232·5°, from *iso*propyl alcohol, of the *dibenzenesulphonate* (Found: N, 11·45, 11·3; S, 10·3. $C_{29}H_{31}O_6N_5S_2$ requires N, 11·5; S, 10·5%) and from the mother-liquors a more soluble salt, crystallising from *iso*propyl alcohol as white plates identified as *N*-4-diphenylylguanidine benzenesulphonate, m. p. 170° (Found: C, 61·3; H, 5·0; S, 8·95. Calc. for $C_{19}H_{19}O_3N_3S$: C, 61·75; H, 5·15; S, 8·7%), undepressed on admixture with an authentic specimen (m. p. 170—171°). The triazine also gave a *dipicrate*, crystallising from aqueous ethanol as needles, m. p. 244—245° (Found: N, 20·1. $C_{32}H_{25}O_{14}N_{11}$ requires N, 20·5%).

A solution of the diguanide benzenesulphonate (m. p. 214°) in methanol was treated with methanolic potassium hydroxide till alkaline to brilliant-yellow, the solution evaporated to dryness, and the residue extracted with boiling chloroform, affording quantitative conversion into N′-4-*diphenylyldiguanide*, crystallising from chloroform–light petroleum or aqueous alcohol as white plates, m. p. 189° (decomp.) (Found: N, 27·1. $C_{14}H_{15}N_5$ requires N, 27·65%). The *picrate* separated from aqueous alcohol in yellow needles, m. p. 203·5—204° (Found: N, 23·7. $C_{20}H_{18}O_7N_8$ requires N, 23·25%).

p′-*Substituted* 4-*Benzylideneaminodiphenyls*.—Formed in quantitative yield by refluxing an alcoholic solution containing equivalent quantities of 4-aminodiphenyl and the substituted benzaldehyde for 0·25 hour, the Schiff's *bases* were recrystallised from alcohol or acetone. Their properties are given in Table I.

p′-*Substituted* 4-*Benzylaminodiphenyls*.—The Schiff's bases (*ca.* 2 g.) described above were reduced by shaking them in ethyl acetate (*ca.* 100 c.c.) with hydrogen in presence of Adams's catalyst (*ca.* 0·02 g.) until 1·1 mols. of hydrogen had been absorbed. Filtration and evaporation of the filtrate afforded a theoretical yield of the *bases*, which were recrystallised from alcohol or ethyl acetate (see Table II). Their *hydrochlorides* (Table II) were prepared by the action of dry hydrogen chloride in ether.

We acknowledge our indebtedness to Dr. W. F. Short for his fundamental work on the chemistry of amidines (*J.*, 1946, 147, and subsequent papers), which made possible the preparation of the substances described in Parts I and II of this series. The present work was carried out under the auspices of the National Health and Medical Research Council, to whom thanks are offered for financial assistance. The authors also thank Mrs. E. Bielski for microanalyses.

UNIVERSITY OF SYDNEY. [*Received, March 20th*, 1951.]

521. *The Chemistry of Fungi. Part XVI.* Eburicoic Acid.*

By R. M. Gascoigne, J. S. E. Holker, B. J. Ralph, and Alexander Robertson.

From the mycelium of the following fungi grown on a synthetical medium a triterpene acid, $C_{30}H_{48}O_3$, eburicoic acid, has been isolated—*Polyporus anthracophilus* Cooke, *P. eucalyptorum* Fr., *P. sulphureus* (Bull) Fr., *Fomes officinalis* Fr., and *Lentinus dactyloides* Clel. The acid from the last two mentioned sources was accompanied by a compound of the same type, containing a conjugate system of double bonds, whilst from mycelium of *P. anthracophilus* Cooke grown naturally on *Eucalyptus regnans* wood the acetyl derivative of eburicoic acid was isolated.

Eburicoic acid is a triterpenoid hydroxy-acid having a tertiary carboxyl group and two double bonds, of which one is easily hydrogenated and is present in a methylene group since on ozonolysis *O*-acetyleburicoic acid gives formaldehyde. In confirmation of this, scission of the diol formed from methyl *O*-acetyleburicoate gave rise to a compound believed to be a ketone along with formaldehyde. Eburicoic acid was converted into the hydrocarbon eburicene-I by a series of standard reactions. Similarly, it appears that the hydroxyl group is located in a terminal ring system and is in the α-position to the carbon atom carrying the *gem*-dimethyl group. It is concluded that eburicoic acid probably belongs to the tetracyclic series of triterpenes.

In the course of studies at present in progress in these laboratories on the metabolic products of a considerable number of the higher fungi, mainly of the class Basidiomycetes, grown on a synthetical medium, a sample of *Eucalyptus regnans* wood rotted by the fungus *Polyporus anthracophilus* Cooke was examined. From the mass of mycelial hyphæ encompassing the decayed wood the acetyl derivative of a monobasic acid, m. p. 292°, was isolated (*cf.* Gascoigne *et al.*, *Nature* 1950, **166**, 652; 1951, **167**, 570) which, from its properties and composition, appeared to belong to the triterpene class. When the fungus responsible for the rot was isolated and grown on a synthetical medium the mycelium contained only the parent acid and not the expected acetate. A preliminary investigation of the acid and some of its derivatives (*loc. cit.*) indicated that the compound had the empirical formula $C_{27}H_{44}O_3$, but, from a subsequent more detailed examination of the compound together with its derivatives and degradation products described in the present communication, it became clear that the acid had the formula $C_{30}H_{48}O_3$. From an examination of the literature it appeared that since the methyl esters and their acetates had similar melting points this acid might be identical with an acid, $C_{30}H_{48}O_3$, m. p. 283°, obtained by Kariyone and Kurono (*J. Pharm. Soc. Japan*, 1940, **60**, 110, 318) from the naturally grown fungus *Fomes officinalis* Fr., which these authors had named eburicoic acid. Accordingly the fungus *Fomes officinalis* Fr. was cultivated on the standard medium and from the mycelium an acid was isolated which differed from the product

	Kariyone and Kurono's acid.		Acid from *P. anthracophilus* Cooke.	
	M. p.	$[\alpha]_D$.	M. p.	$[\alpha]_D$.
Acid	283°	—	292°	—
Methyl ester	141	+37·2°	140—141	+42·4°
Acetate	240	+80	256—257	+48·1
Methyl ester acetate	150	+56·9	154—155	+48·7

of *P. anthracophilus* Cooke in showing selective absorption in the ultra-violet with a peak at 243 mμ. and associated subsidiary peaks at 236 mμ. and 251 mμ. From the low intensity of the absorption, $E^{1\%}_{1\,cm.}$ 37·8, it appeared that the product was a mixture consisting mainly of eburicoic acid along with a small amount of an unsaturated analogue which was extremely difficult to remove. By chromatography of the acetylated mixture on neutralised aluminium oxide with subsequent deacetylation a small amount of an acid, m. p. 292°, was obtained which was identical in every way with the acid obtained from decayed *E. regnans* and from the mycelium of artificially cultivated *P. anthracophilus*. Accordingly, therefore, we propose to retain the name eburicoic acid for this compound but are at present unable to say whether the product described by the Japanese workers contained the unsaturated impurity. It is of

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interest to note that the naturally grown *Fomes officinalis* Fr. is a source of agaracinic acid but when grown on the synthetical medium the fungus does not form this product. As Kariyone and Kurono (*loc. cit.*) employed a hydrolytic process in the isolation of their product it is not clear whether the eburicoic acid contained in it was originally present in the free state or as the acetate. In this connection it may be noted that numerous earlier workers have examined natural *Fomes officinalis* Fr. and on a number of occasions less pure forms of the product obtained by Kuriyone and Kurono have been described (*cf.* Berzelius, " Lehrbuch der Chemie," 1838, **7**, 448; Thom and Vogelsang, *Annalen*, 1907, **357**, 145).

In the examination of the metabolic products of other Basidiomycetes grown on the standard medium, eburicoic acid has been isolated from *Polyporus eucalyptorum* Fr., *P. sulphureus* (Bull) Fr. (*cf.* Zellner and Zikmunda, *Monatsh.*, 1930, **56**, 200, who obtained a compound, m. p. 265°, from the naturally grown fungus, which may have been the impure acid), and *Lentinus dactyloides* Clel. The acid from the last-mentioned fungus also contained the unsaturated contaminant ($E^{1\%}_{1\,cm.}$ at 243 mμ. ranging from 99 to 125 in different batches) which was removed by chromatography. The value of the $\lambda_{max.}$ together with the structure of the ultra-violet absorption curve of the mixture clearly indicates that the contaminant contains a conjugate system of double bonds (*cf.* Booker *et al.*, *J.*, 1940, 1453, and Woodward, *J. Amer. Chem. Soc.*, 1942, **64**, 72), and work in progress indicates that this compound is closely related to eburicoic acid. The difficulties encountered in separating the natural mixture are reminiscent of the non-separable mixtures of lanosterol and agnosterol with their dihydro-compounds (Ruzicka *et al.*, *Helv. Chim Acta*, 1945, **28**, 759; 1946, **29**, 204).

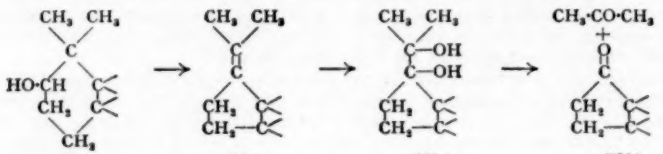
Since eburicoic acid is not esterified by the application of the Fischer–Speier method, and the methyl ester formed with diazomethane is not readily hydrolysed, a property observed by Kariyone and Kurono (*loc. cit.*), it seems likely that the carboxyl group is tertiary. From the behaviour of methyl *O*-acetyleburicoate with perbenzoic acid it is clear that the parent acid contains two double bonds, of which one is easily hydrogenated, giving rise to dihydroeburicoic acid; the second double bond appears to resist hydrogenation. The reactive double bond is clearly present in a methylene group since on ozonolysis *O*-acetyleburicoic acid gave a comparatively good yield of formaldehyde whereas *O*-acetyldihydroeburicoic acid furnished only insignificant amounts. The main ozonolysis product of *O*-acetyleburicoic could not be crystallised but when methyl *O*-acetyleburicoate was oxidised with osmium tetroxide and the resulting diol, $C_{33}H_{54}O_6$, submitted to the action of lead tetra-acetate, formaldehyde was formed along with a crystalline compound believed to be the ketone, $C_{32}H_{50}O_5$, the absorption spectrum of which indicated that it was not an αβ-unsaturated ketone. With the presence of two double bonds in the molecule it seems that eburicoic acid belongs to the tetracyclic triterpene series, and a number of its properties are similar to those of well-known members of this group—lanosterol, agnosterol, elemadienolic acid, and euphol. From preliminary experiments on the dehydrogenation of eburicoic acid with selenium the main hydrocarbon formed appears to be a tri- or tetra-methylphenanthrene, a result typical of the tetracyclic group. It differs, however, from these compounds in yielding formaldehyde and not acetone on ozonolysis but in this respect it resembles basseol and the other triterpenoid acids isolated from fungi, *viz.* the polyporenic acids which also contain one inert and one reactive double bond (Cross *et al.*, *J.*, 1940, 632, 1491; Morgan, *Biochem. J.*, 1951, **48**, xliii). Eburicoic acid, however, does not cyclise under the conditions by which basseol is converted into β-amyrin.

In an attempt to relate eburicoic acid more closely to known triterpenes it was converted into the hydrocarbon eburicene-I.* By Oppenauer's method with *cyclo*hexanone in dioxan as the oxidising agent, eburicoic acid yielded a keto-acid, eburicodienonic acid, the absorption spectrum of which (inflexion at 282 mμ., log ε 1·5) indicates that the keto-group was not conjugated. By reduction according to the Wolff–Kishner method the hydrazone of this keto-acid gave eburicodienic acid and on hydrogenation the methyl ester of this compound furnished methyl eburicenate which with lithium aluminium hydride yielded the alcohol eburicenol. With *p*-benzoquinone as the oxidising agent the oxidation of eburicenol according to Oppenauer gave rise to the aldehyde eburicenal, the semicarbazone of which was reduced by the Wolff–Kishner method to eburicene-I. When, however, the semicarbazone was replaced by the hydrazone in this reaction eburicene-I was accompanied by a small amount of an isomeric hydrocarbon eburicene-II, neither of which appears to be identical with any of the known triterpenoid hydrocarbons.

* The rational nomenclature is based on the name eburicane for the hypothetical fully saturated hydrocarbon $C_{30}H_{54}$. Thus eburicoic acid becomes eburicodienolic acid and its dihydro-derivative eburicenolic acid.

In the exploration of the nature of eburicoic acid the compound was submitted to the following series of reactions which have been used to elucidate the structure of ring A in several well-known triterpene compounds. Dehydration of methyl dihydroeburicoate (I) with phosphorus pentachloride gave methyl eburicodienate-II (II), and with osmium tetroxide this compound furnished a diol (III) which on oxidation with lead tetra-acetate gave a high yield of acetone along with a ketone (IV). Although this ketone did not crystallise, the well-defined semicarbazone and 2 : 4-dinitrophenylhydrazone served to establish clearly its formula as $C_{28}H_{44}O_3$. The absorption spectrum of the semicarbazone ($\lambda_{max.}$ 225 mμ.; log ε 4·2) indicated that the keto-group was not conjugated. In this connection it may be noted that the absorption spectrum of methyl eburicotrienate, formed by the dehydration of methyl eburicoate, indicated the absence of a conjugated system.

The formation of acetone from methyl eburicodienate-II in the foregoing manner suggests that the dehydration of methyl dihydroeburicoate proceeds in the same way as in a number of pentacyclic triterpenoids and also in the tetracyclic lanosterol (Ruzicka *et al.*, *Helv. Chim. Acta*, 1948, **31**, 818; Dorée *et al.*, *J.*, 1949, S167) where in the dehydration process a retropinacolic rearrangement is postulated resulting in the contraction of the ring originally containing the hydroxyl group, with the formation of an *iso*propylidene group :



(I.) (II.) (III.) (IV.)

This rearrangement requires that the hydroxyl group is located in a terminal ring in the α-position to the carbon atom carrying the *gem*-dimethyl group.

Experimental.

The melting points given are uncorrected and all analytical specimens were dried to constant weight in a high vacuum at elevated temperatures. Alcoholic solutions of the compounds were employed for the absorption-spectra measurements.

O-Acetyleburicoic Acid from Eucalyptus regnans.—A sample of decayed wood of *Eucalyptus regnans* which was kindly supplied to us by Mr. J. Somerville of the Australian Newsprint Mills, Boyer, consisted of a fine white powder containing fragments of wood and fungus mycelium. This was extracted with boiling alcohol and on cooling the concentrated extract gave *O*-acetyleburicoic acid in clusters of long silky needles which on recrystallisation had m. p. 256—257°, $[\alpha]_D^{25}$ +33·4° (*c*, 3·1 in pyridine) and was soluble in the usual organic solvents except light petroleum (Found : C, 77·0; H, 10·0. Calc. for $C_{32}H_{50}O_4$: C, 77·0; H, 10·1%). Deacetylation of *O*-acetyleburicoic acid with boiling 10% alcoholic potassium hydroxide yielded eburicoic acid which crystallised from alcohol in felted masses of slender needles, m. p. 292° (Found : C, 78·9; H, 10·5. Calc. for $C_{30}H_{48}O_3$: C, 78·9; H, 10·6%). Prepared with ethereal diazomethane, methyl *O*-acetyleburicoate formed silky needles, m. p. 154—155°, from methanol [Found : C, 77·3; H, 10·3; OMe, 5·7. Calc. for $C_{33}H_{52}O_5$(OMe) : C, 77·3; H, 10·2; OMe, 6·0%]. On being boiled with 10% methanolic potassium hydroxide (50 ml.) for 1 hour this compound (0·2 g.) gave methyl eburicoate which separated from methanol in slender needles, m. p. 140—141° [Found : C, 79·2; H, 10·6; OMe, 6·9. Calc. for $C_{30}H_{47}O_3$(OMe) : C, 79·1; H, 10·7; OMe, 6·6%].

Perbenzoic Acid Titration of Methyl Acetyleburicoate.—To the ester (99·5 mg.), dissolved in pure chloroform (20 ml.), at 0° a solution of perbenzoic acid in chloroform (5 ml.; 0·5N.) was added. The mixture was kept at 0° and samples withdrawn and treated with a mixture of potassium iodide (0·5 g.) and acetic acid (3 ml.) in water (20 ml.) which was then titrated with 0·1005N-sodium thiosulphate solution, starch indicator being used. A blank determination was carried out at the same time and under the same conditions. After 1 day 1·97 equivalents and after 2 days 2·18 equivalents of perbenzoic acid were consumed; the latter did not change after 7 days.

Eburicoic Acid from Fungi Cultivated on a Synthetical Medium.—The following fungi were grown on a modified Williams–Saunders medium (*Biochem. J.*, 1934, **28**, 1887) containing " Marmite " (0·1 g. per litre) with glucose (100 g. per litre) in place of sucrose and glycine (2 g. per litre) in place of asparagine. The identification of the cultures was checked for us by Professor J. Westerdijk of the Centraalbureau voor Schimmelcultures, Baarn, to whom our thanks are due.

The washed mycelium of each species was dried in a vacuum at 50°, milled to a fine powder, and extracted in a Soxhlet, first with light petroleum (b. p. 40—60°) to remove fat and then exhaustively with ether. The residue left on evaporation of the ethereal extract was purified by repeated crystallisation from alcohol until a product of constant melting point was obtained. The yields of eburicoic

acid given are those of once crystallised material and are expressed as a percentage of the weight of dried mycelium.

(a) *From* Polyporus anthracophilus *Cooke*. The product (yield *ca.* 10%) was contaminated with a small amount of a low-melting non-acidic compound which could not be satisfactorily removed by recrystallisation from alcohol. Consequently the acid was converted into the sodium salt (see below) which after having been extracted several times with boiling benzene was decomposed, giving eburicoic acid which crystallised from alcohol in colourless slender needles, m. p. 292°, $[\alpha]_D^{29}$ +35·9° (*c*, 1·8 in pyridine) (Found: C, 78·8; H, 10·4. Calc. for $C_{30}H_{48}O_3$: C, 78·9; H, 10·6%), moderately soluble in polar solvents and slightly soluble in non-polar solvents. In the Liebermann–Burchard reaction the acid gave a stable ruby-red coloration. The Salkowski and Rosenheim tests with the acid were negative. The *sodium* salt was prepared by treatment of the pure or partially purified acid with a hot mixture of equal volumes of 2N-aqueous sodium hydroxide and alcohol, followed by the addition of water. The cooled mixture deposited the salt in long glistening needles which were recrystallised from warm aqueous alcohol containing a trace of sodium hydroxide (Found: Na, 4·9, 4·7. $C_{30}H_{47}O_3Na$ requires Na, 4·8%). This compound is insoluble in water but is readily soluble in alcohol.

On being heated with acetic anhydride–pyridine on the steam-bath for 3 hours, the acid or its sodium salt gave rise to *O*-acetyleburicoic acid which separated from dilute alcohol in long silky needles, m. p. 256—257°, $[\alpha]_D^{20}$ +33·6° (*c*, 3·0 in pyridine), $[\alpha]_D^{21}$ +48·1° (*c*, 2·3 in chloroform), identical with the natural compound (Found: C, 76·7; H, 10·4. Calc. for $C_{32}H_{50}O_4$: C, 77·0; H, 10·1%).

Prepared with ethereal diazomethane, methyl eburicoate crystallised from methanol in slender needles, m. p. 140—141°, $[\alpha]_D^{22}$ +33·1° (*c*, 3·4 in pyridine), $[\alpha]_D^{17}$ +42·4° (*c*, 2·7 in chloroform) (Found: C, 78·6; H, 11·0. Calc. for $C_{31}H_{50}O_3$: C, 79·1; H, 10·7%). The methyl ester was also obtained by heating sodium eburicoate with methanolic methyl iodide for several hours and was isolated from the evaporated reaction mixture with benzene. This ester, which could not be prepared by the Fischer–Speier method, did not appear to be readily hydrolysed on being boiled with 10% alcoholic potassium hydroxide; after treatment for 2 hours 96% of the unchanged ester was recovered with only a trace of acid.

The acetate of methyl eburicoate separated from methanol in silky needles, m. p. 154—155°, $[\alpha]_D^{22}$ +30·8° (*c*, 3·1 in pyridine), $[\alpha]_D^{19}$ +48·7° (*c*, 2·4 in chloroform) (Found: C, 77·3; H, 10·1. Calc. for $C_{33}H_{52}O_4$: C, 77·3; H, 10·2%).

On being heated under reflux for 2 hours a mixture of sodium eburicoate (0·25 g.) and *p*-nitrobenzyl bromide (0·12 g.) in alcohol (15 ml.) gave rise to *p-nitrobenzyl eburicoate* which crystallised from alcohol in long colourless needles, m. p. 150—150·5° (Found: C, 74·8; H, 9·2; N, 2·5. $C_{37}H_{53}O_5N$ requires C, 75·0; H, 9·0; N, 2·4%).

(b) *From* Polyporus eucalyptorum *Fr.* The acid (yield 20%) had m. p. 293°, $[\alpha]_D^{17}$ +35·6° (*c*, 1·4 in pyridine) (Found, C, 79·0, 78·8; H, 10·6, 10·6%), and gave the methyl ester, m. p. 140—141° (Found: C, 79·1; H, 10·5%), the *p*-nitrobenzyl ester, m. p. 150° (Found: C, 75·2; H, 8·9; N, 2·4%), and the acetate, m. p. 254°, $[\alpha]_D^{25}$ +35·8° (*c*, 3·1 in pyridine) (Found: C, 77·0; H, 10·0%). Methyl *O*-acetyleburicoate had m. p. 153—154° (Found: C, 77·5; H, 10·3%).

(c) *From* Polyporus sulphureus (*Bull*) *Fr.* The acid (yield 10%) had m. p. 293°, $[\alpha]_D^{20}$ +38·6° (*c*, 5·1 in pyridine) (Found: C, 79·1; H, 10·4%), and gave the acetate, m. p. 253—255°, $[\alpha]_D^{19}$ +33·8° (*c*, 4·6 in pyridine) (Found: C, 76·8; H, 10·5%), the methyl ester, m. p. 140—141° (Found: C, 78·9; H, 10·8%), and the acetate of the methyl ester, m. p. 154—155° (Found: C, 77·1; H, 10·4%).

(d) *From* Fomes officinalis *Fr.* The crystalline acidic product (yield 6%) from this mould had m. p. 293° and gave an acetate, m. p. 254°, but differed from eburicoic acid from the sources (*a*), (*b*), and (*c*) in showing absorption in the ultra-violet (maximum at 243 mμ., $E_{1\,cm.}^{1\%}$ 37·8). The acetate (6·45 g.) of this product was chromatographed from benzene–light petroleum (b. p. 60—80°) (1 l. of 1 : 4 mixture) on a column of alumina (60 cm. × 6 cm.; 1300 g.) which had been neutralised by being washed with methanol containing 10% of acetic acid and then several times with methanol, and dried at 120°. The column was eluted with benzene (3 l.), which removed only a trace of material, and then with 10% acetone in benzene. This gave a fraction (0·24 g.), $E_{1\,cm.}^{1\%}$ 8·5 at 243 mμ., consisting of *O*-acetyleburicoic acid, which on recrystallisation from dilute alcohol had m. p. 256—257°, $[\alpha]_D^{25}$ +31·9° (*c*, 2·2 in pyridine) (Found: C, 76·9; H, 10·4%). With ethereal diazomethane it gave methyl *O*-acetyleburicoate, m. p. 154—155° (Found: C, 76·9; H, 10·5%), and on hydrolysis furnished eburicoic acid, m. p. 292—293°, $[\alpha]_D^{22}$ +35·1° (*c*, 1·6 in pyridine) (Found: C, 78·9; H, 10·7%), giving the methyl ester, m. p. 140° (Found: C, 78·8; H, 11·2%), and the *p*-nitrobenzyl ester, m. p. 151—152° (Found: C, 74·8; H, 9·1; N, 2·7%). Subsequent elution of the chromatogram with 50% acetone in benzene gave a series of fractions, of increasing $E_{1\,cm.}^{1\%}$, which have been reserved for detailed examination.

(e) *From* Lentinus dactyloides *Clel.* The acidic fraction (yield *ca.* 5%) from the mycelium of this mould had m. p. 285° after repeated recrystallisation, with $E_{1\,cm.}^{1\%}$ at 243 mμ. ranging from 99 to 125 in different batches. The mixed acetates (7 g.) were chromatographed on neutralised alumina [see (*d*)] (112 cm. × 1·8 cm.; 280 g.) from 700 ml. of 1 : 4-benzene–light petroleum (b. p. 60—80°), and the column was washed with a further 300 ml. of mixed solvent and allowed to drain. The filtrate contained only minute traces of material. The column was then divided into sections and each section eluted with hot methanol (250 ml. × 10), and the extracts evaporated. The bottom 5-cm. portion gave only a trace of material. The next and the subsequent 5-cm. portion gave solid products (0·12 g., $E_{1\,cm.}^{1\%}$ 6·3 at 243 mμ., and 0·24 g., $E_{1\,cm.}^{1\%}$ 6·6 at 243 mμ., respectively) which were combined and on recrystallisation from dilute alcohol gave *O*-acetyleburicoic acid, m. p. 255—256°, $[\alpha]_D^{23}$ +36·4° (*c*, 1·7

in pyridine) (Found : C, 76·8; H, 10·3%), which was converted into methyl *O*-acetyleburicoic acid, m. p. 154—155° (Found : C, 76·8; H, 10·4%). On deacetylation the acetate gave eburicoic acid, m. p. 292—293°, $[\alpha]_D^{23}$ +34·8° (*c*, 1·5 in pyridine) (Found : C, 78·9; H, 10·4%), which formed the methyl ester, m. p. 140° (Found : C, 78·8; H, 11·0%), and the *p*-nitrobenzyl ester, m. p. 151—152° (Found : C, 75·3; H, 8·9; N, 2·6%).

The remaining sections of the column yielded products in which the value of $E_{1\,cm}^{1\%}$ at 243 mμ. increased to a value of 110—115 at the top of the column.

The equivalent weight of eburicoic acid was determined by the procedure described by Ruzicka and Furter (*Helv. Chim. Acta*, 1932, **15**, 472), and the alkali employed was standardised against oleanolic acid. Thus eburicoic acid from *Polyporus anthracophilus* Cooke had equiv. 459, 462, from *P. eucalyptorum* Fr., equiv. 459, 461, and from *P. sulphureus* (Bull) Fr., equiv. 463, 465 (theoretical for $C_{30}H_{48}O_3$: equiv. 457). Betulic acid gave equiv. 457, 463 by same method.

Dihydroeburicoic Acid.—Hydrogenation of eburicoic acid (1·2 g.), dissolved in alcohol (250 ml.), with hydrogen (approx. 1 mol. absorbed) at atmospheric pressure and a palladium–charcoal catalyst (from 0·1 g. of palladium chloride and 1 g. of charcoal) was complete in one hour. On isolation the resulting *dihydroeburicoic acid* crystallised from alcohol in long needles, m. p. 295—297° (Found : C, 78·5; H, 10·9. $C_{30}H_{50}O_3$ requires C, 78·4; H, 11·0%). The *acetate* separated from dilute alcohol in long silky needles, m. p. 272—274°, giving a yellow coloration with tetranitromethane in chloroform (Found : C, 76·9; H, 10·4. $C_{32}H_{52}O_4$ requires C, 76·8; H, 10·5%), and the *methyl* ester from methanol in glistening prisms, m. p. 132° (Found : C, 78·6; H, 11·0. $C_{31}H_{52}O_3$ requires C, 78·7; H, 11·1%). *Methyl* O-*acetyldihydroeburicoate* formed stout prisms, m. p. 157—159° (Found : C, 76·9; H, 10·6. $C_{33}H_{54}O_4$ requires C, 76·9; H, 10·6%). Attempts to prepare a tetrahydro-derivative of eburicoic acid by means of an active platinum catalyst and hydrogen at 150°/100 atmospheres' pressure gave only the dihydro-compound.

Ozonolysis of O-*Acetyleburicoic Acid.*—A stream of ozone and oxygen was led into a solution of the acetate (1 g.) in chloroform (100 ml.) at 0° for 4 hours. After the evaporation of the solvent in a vacuum at room temperature the vitreous residue was treated with water (200 ml.), and 12 hours later the mixture was warmed on the steam-bath for ½ hour. Formaldehyde was removed from the reaction mixture by a current of steam, giving the dimedone derivative in needles, m. p. 189°, identified by comparison with an authentic specimen; the yield in several experiments ranged from 0·17 g. to 0·24 g., *i.e.*, 29% to 41% of the theoretical for one methylene group. In another experiment the formaldehyde was converted into the 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 162° after purification. In addition to formaldehyde, a colourless amorphous solid was obtained which gave an amorphous dinitrophenylhydrazone.

Ozonolysis of *O*-acetyldihydroeburicoic acid (1 g.) under the same conditions gave rise to only a trace (11 mg.) of the dimedone derivative of formaldehyde.

Oxidation of Methyl O-*Acetyleburicoate with Osmium Tetroxide.*—The oxide (1 g.) was added to a solution of the ester (3·72 g.) in ether (70 ml.), and on being kept for 24 hours the mixture deposited the osmic ester as a black solid. A solution of this product (2·27 g.), which had been washed with ether until the washings were colourless, in methanol (200 ml.) was added to aqueous sodium sulphite (30 g. in 150 ml. of water), and the mixture was heated under reflux for one hour and filtered. The solid was extracted with boiling methanol (3 × 100 ml.) and the combined filtrates and washings were evaporated in a vacuum. On concentration a methanolic extract of the residue gave a *diol* in small prisms (1·18 g.) which on recrystallisation from methanol had m. p. 195—196·5° (Found : C, 72·2; H, 10·2. $C_{33}H_{54}O_6$ requires C, 72·4; H, 9·9%).

Treatment of this diol (963 mg.), dissolved in acetic acid (8 ml.), with lead tetra-acetate (915 mg.) for 24 hours gave a *ketone*, part of which separated, the remainder being precipitated with water (50 ml.). On recrystallisation from methanol this compound formed long glistening needles (536 mg.), m. p. 177—178°; absorption spectrum $\lambda_{max.}$ 280 mμ., log ε 1·84 (Found : C, 74·6; H, 9·7. $C_{32}H_{50}O_5$ requires C, 74·6; H, 9·8%). The *oxime* separated from methanol in stout prisms, m. p. 171—172° (Found : C, 72·5; H, 9·8; N, 2·7. $C_{32}H_{51}O_5N$ requires C, 72·5; H, 9·7; N, 2·6%), and the *semicarbazone* in prisms, m. p. 218—219° (decomp.), absorption spectrum $\lambda_{max.}$ 225 mμ., log ε 4·18 (Found : C, 69·5; H, 9·5; N, 7·7. $C_{33}H_{53}O_5N_3$ requires C, 69·2; H, 9·3; N, 7·3%).

Treatment of the aqueous acetic acid filtrate from the crude ketone with dimedone gave the formaldehyde–dimedone derivative (360 mg.), m. p. and mixed m. p. 189°.

Oppenauer Oxidation of Eburicoic Acid.—A mixture of the acid (10 g.), *cyclo*hexanone (25 ml., 10 mol.), aluminium *tert.*-butoxide (15 g., 2·5 mol.), and dioxan (75 ml.) was heated under reflux for 6 hours, cooled, and poured into 2N-sulphuric acid (100 ml.). The dioxan and excess of *cyclo*hexanone were removed with steam, and the residual solid was crystallised from alcohol, giving *eburicodienonic acid* (8·1 g.) in needles, m. p. 226—227°, $[\alpha]_D^{20}$ +50·9° (*c*, 4·4 in pyridine); the absorption spectra showed an inflexion at 282 mμ. (log ε 1·54) (Found : C, 79·2; H, 10·2. $C_{30}H_{46}O_3$ requires C, 79·1; H, 10·2%). This acid, which is readily soluble in the usual solvents except light petroleum, did not react with Fehling's or Tollens's reagent. The *semicarbazone* separated from alcohol in leaflets, m. p. 313° (decomp.) (absorption spectrum max. at 225 mμ., log ε 4·17) (Found : C, 72·7; H, 9·5; N, 8·3. $C_{31}H_{49}O_3N_3$ requires C, 72·6; H, 9·6; N, 8·2%), and the *oxime* in needles, m. p. 243° (decomp.) (Found : C, 76·8; H, 10·2; N, 3·0. $C_{30}H_{47}O_3N$ requires C, 76·6; H, 10·1; N, 3·0%).

Eburicenonic acid was prepared from dihydroeburicoic acid by the method employed for eburicodienonic acid and formed rosettes of needles, m. p. 238—240° (Found : C, 79·0; H, 10·6. $C_{30}H_{48}O_3$

requires C, 78·9; H, 10·6%). The *semicarbazone* separated from alcohol in needles, m. p. 308—309° (decomp.) (Found: C, 72·5; H, 10·1; N, 8·0. $C_{31}H_{51}O_3N_3$ requires C, 72·4; H, 10·0; N, 8·2%).

Eburicodienic Acid.—A mixture of eburicodienonic acid (1·52 g.), 90% hydrazine hydrate (0·5 ml.), potassium hydroxide (0·7 g.), and diethylene glycol (5 ml.) was heated under reflux for 1½ hours. After the removal of the excess of hydrazine hydrate by distillation the reaction mixture was kept at 195° for 4 hours to decompose the hydrazone (evolution of gas and frothing), cooled, diluted with water (5 ml.), and treated with 6N-hydrochloric acid (6 ml.) followed by ether (2 ml.). A solid product separated and, after the evaporation of the ether, this was collected and crystallised from alcohol, giving *eburicodienic acid* in sheaves of needles (1·1 g.), m. p. 252—253°, $[\alpha]_D^{16}$ +40·5° (*c*, 3·0 in pyridine) (Found: C, 81·8; H, 11·1. $C_{30}H_{48}O_2$ requires C, 81·7; H, 11·0%). Prepared by use of ethereal diazomethane, the *methyl* ester formed needles, m. p. 98—99°, from methanol–chloroform (Found: C, 81·6; H, 11·2. $C_{31}H_{50}O_2$ requires C, 81·8; H, 11·1%), and on hydrogenation with W5 Raney nickel catalyst and hydrogen at atmospheric pressure gave rise to *methyl eburicenate* which crystallised from methanol–chloroform in elongated plates, m. p. 118—119·5° (Found: C, 81·6; H, 11·6. $C_{31}H_{52}O_2$ requires C, 81·4; H, 11·5%).

Eburicenol.—A mixture of methyl eburicenate (5·2 g.), lithium aluminium hydride (3 g.), and ether (150 ml.) was heated under reflux for 3 hours, kept for 14 hours, and poured on crushed ice (200 g.) and 2N-sulphuric acid (50 ml.). After the vigorous reaction had subsided the ethereal layer was isolated, washed, dried, and evaporated, leaving *eburicenol* (4·8 g.) which separated from methanol in rosettes of needles, m. p. 100—102°, $[\alpha]_D^{18}$ +54·5° (*c*, 3·0 in pyridine) (Found: C, 83·7; H, 12·3. $C_{30}H_{52}O$ requires C, 84·0; H, 12·2%). The *acetate* formed needles, m. p. 77—78°, from aqueous alcohol (Found: C, 81·7; H, 12·2. $C_{32}H_{54}O_2$ requires C, 81·6; H, 11·6%), and the *p-nitrobenzoate* needles, m. p. 112—114°, from methanol (Found: C, 76·5; H, 9·6. $C_{37}H_{53}O_4N$ requires C, 76·9; H, 9·6%).

Eburicenal.—A solution of eburicenol (3·8 g.), *p*-benzoquinone (8·8 g.), and aluminium *tert.*-butoxide (6 g.) in benzene (250 ml.) was heated under reflux for 14 hours. 2N-Sulphuric acid (50 ml.) was added to the cooled mixture, and the benzene and excess of quinone removed with steam. An ethereal solution of the reaction product was well washed with 2N-aqueous sodium hydroxide to remove the brown material and then with water, and dried, and evaporated, leaving a buff solid (3·2 g.); crystallised from alcohol–benzene, this gave *eburicenal* in colourless plates, m. p. 157—158°, $[\alpha]_D^{23}$ +53·1° (*c*, 3·0 in pyridine) (Found: C, 84·2; H, 12·0. $C_{30}H_{50}O$ requires C, 84·4; H, 11·8%). The *semicarbazone* separated from alcohol in short stout needles, m. p. 257—258° (decomp.) (Found: C, 76·9; H, 11·2; N, 8·8. $C_{31}H_{53}ON_3$ requires C, 76·9; H, 11·0; N, 8·7%).

Reduction of Eburicenal.—(*a*) A mixture of the semicarbazone (930 mg.) of eburicenal in alcohol (40 ml.), containing sodium ethoxide (from 1·8 g. of sodium), was kept at 190—200° for 12 hours, cooled, diluted with water, and acidified with dilute sulphuric acid. Isolated with ether, the product was a yellow oil (800 mg.) which was resolved into a main fraction (640 mg.), a colourless oil which solidified, and small amounts (80 mg. and 20 mg.) of bright yellow oils, by chromatography on alumina from light petroleum (b. p. 60—80°). On recrystallisation from alcohol–benzene, the solid fraction gave *eburicene*-I in colourless plates, m. p. 122—123°, $[\alpha]_D^{16}$ +55·0° (*c*, 2·0 in chloroform) (Found: C, 87·4; H, 12·6. $C_{30}H_{52}$ requires C, 87·3; H, 12·7%).

(*b*) A solution of eburicenal (770 mg.), potassium hydroxide (700 mg.), 90% hydrazine hydrate (0·5 ml.), and diethylene glycol (5 ml.) was heated under reflux for 1½ hours. The excess of hydrazine was removed by distillation and the mixture then kept at 195° for 4 hours, cooled, diluted with water, and acidified with dilute sulphuric acid. On isolation with ether the product was a yellow oil (600 mg.) which was resolved by chromatography from light petroleum (b. p. 60—80°) on alumina into two main and two subsidiary fractions. Fraction (1) (280 mg.) consisted of eburicene-I, m. p. and mixed m. p. 122—123° after recrystallisation from alcohol–benzene (Found: C, 87·3; H, 12·8%). Fraction (2) consisted of a colourless solid which on recrystallisation from alcohol–benzene gave *eburicene*-II in felted needles, m. p. 274—275°, $[\alpha]_D^{20}$ +51·3° (*c*, 0·8 in chloroform) (Found: C, 87·2, 87·1; H, 12·7, (12·7%. Fractions (3) (20 mg.) and (4) (100 mg.) were yellow oils which were not investigated.

Methyl Eburicotrienate.—A mixture of methyl eburicoate (1 g.), phosphorus pentachloride (0·75 g.), and light petroleum (b. p. 40—60°) (150 ml.) was vigorously agitated for 45 minutes and then kept for 24 hours. The light petroleum solution was washed with water (2 × 50 ml.), 2N-aqueous sodium hydroxide (2 × 50 ml.), and again with water (2 × 50 ml.), and dried and evaporated. Crystallisation of the residue from methanol gave *methyl eburicotrienate* in fine needles which in contact with the solvent changed to prisms (0·5 g.), m. p. 115—116° (Found: C, 82·6; H, 10·6. $C_{31}H_{48}O_2$ requires C, 82·2; H, 10·7%).

By the same method methyl dihydroeburicoate was converted into *methyl eburicodienate*-II which formed rectangular plates, m. p. 137—138°, from methanol (Found: C, 81·9; H, 10·9. $C_{31}H_{50}O_2$ requires C, 81·8; H, 11·1%).

Oxidation of Methyl Eburicodienate-II.—A mixture of methyl eburicodienate (696 mg.), osmium tetroxide (426 mg.), and ether (10 ml.) was kept for 24 hours, and the resulting black solid washed with cold ether until the washings were colourless. A solution of the residue (877 mg.) in methanol (50 ml.) was treated with sodium sulphite (10 g.; dissolved in 50 ml. of water), heated under reflux for 1 hour, and then filtered and the solid washed with methanol (50 ml.). The combined filtrate and washings were evaporated and the residue extracted with boiling methanol. On concentration the extract gave the *diol* in needles (361 mg.), m. p. 158—159°, after recrystallisation from methanol (Found: C, 76·3; H, 10·7. $C_{31}H_{52}O_4$ requires C, 76·2; H, 10·7%).

Oxidation of this diol (800 mg.) with lead tetra-acetate (800 mg.) in acetic acid (7 ml.) during 12 hours followed by dilution of the reaction mixture with water gave rise to a gum which did not crystallise. This product gave a *semicarbazone* forming, from methanol, rosettes of needles, m. p. 250°; light

absorption, maximum 223 mμ., log ε 4·25 (Found: C, 71·8; H, 9·8; N, 8·8. $C_{22}H_{47}O_3N_3$ requires C, 71·7; H, 9·8; N, 8·7%). The 2 : 4-*dinitrophenylhydrazone* was purified by chromatography from benzene on alumina and then by crystallisation from methanol, forming orange needles, m. p. 225° (decomp.) (Found: C, 66·5; H, 8·1; N, 9·6. $C_{34}H_{48}O_6N_4$ requires C, 67·1; H, 8·0; N, 9·2%). Attempts to crystallise specimens of the ketone regenerated from the semicarbazone or from Girard reagent P were unsuccessful.

Acetone was isolated by distillation from the aqueous acetic acid liquor left after the separation of the crude ketone and was converted into the 2 : 4-dinitrophenylhydrazone, m. p. 125—126°, after purification from alcohol; yield, 70% of theoretical (Found: C, 45·5; H, 4·3; N, 23·4. Calc. for $C_9H_{10}O_4N_4$: C, 45·4; H, 4·2; N, 23·6%).

UNIVERSITY OF LIVERPOOL. [*Received, March 28th*, 1951.]

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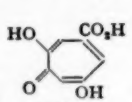
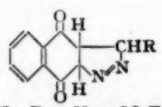
522. *A Synthesis of Stipitatic Acid.*

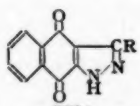
By J. R. BARTELS-KEITH, A. W. JOHNSON, and W. I. TAYLOR.

A method of synthesis of the tropolone ring has been developed which involves a ring expansion of veratroles with diazoacetic ester and subsequent oxidative hydrolysis of the products. Syntheses of tropolone-β-carboxylic acid, and therefore of tropolone itself, and of stipitatic acid are described, as well as several derivatives of the acids.

IN an earlier paper (Corbett, Johnson, and Todd, *J.*, 1950, 147), the correctness of the tropolone formulation (I) for stipitatic acid (Birkinshaw, Chambers, and Raistrick, *Biochem. J.*, 1942, **36**, 242), as originally advanced by Dewar (*Nature*, 1945, **155**, 50), was established unequivocally on degradative evidence. At that time no tropolone syntheses had been described, and after a number of unsuccessful approaches the reaction between diazoacetic ester and methoxybenzoquinone was investigated, which, if a ring enlargement occurred, should have given a methyl ether of stipitatic acid directly. The reaction between 1 : 4-quinones and diazoacetic ester has been investigated previously only in the case of 1 : 4-naphthaquinone (Fieser and Peters, *J. Amer. Chem. Soc.*, 1931, **53**, 4080), although there are several examples of the reaction of quinones with diazomethane (*inter alia*: Fieser and Peters, *loc. cit.*; Wallenfels, *Ber.*, 1942, **75**, 787; Spruit, *Rec. Trav. chim.*, 1947, **66**, 655; 1949, **68**, 304). The course of these earlier reactions was not encouraging from the point of view of synthesis of tropolone; the first-formed pyrazoline-quinones [*e.g.*, (II) from 1 : 4-naphthaquinone] rearranged to the pyrazole-quinols, which were oxidised to the corresponding quinones (*e.g.*, III). The final products did not evolve nitrogen when heated.


(I.)


(II; R = H or CO_2Et.)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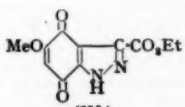
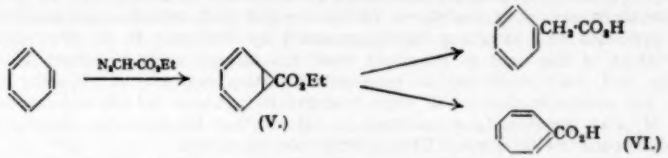

(IV.)

The reaction between methoxybenzoquinone and diazoacetic ester exactly paralleled that with 1 : 4-naphthaquinone, the product (IV), 5 (or 6)-methoxyindazole-4 : 7-quinone-3-carboxylic ester, being pale yellow and not evolving nitrogen even at 200°. It is well known that the presence of substituents on the nuclear carbon atoms of the quinone diminish the ease of addition reactions to the double bonds, and there can be little doubt that the addition of diazoacetic ester occurs at the unsubstituted double bond of methoxybenzoquinone as indicated.

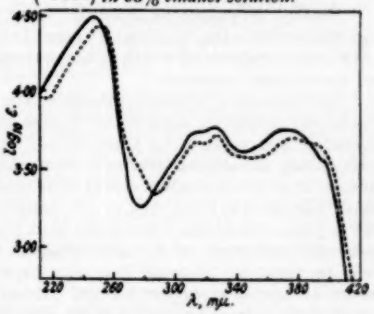
Attention was therefore turned to the reaction of diazoacetic ester with methoxyquinol in the form of its dimethyl ether, 1 : 2 : 4-trimethoxybenzene, and as a preliminary the corresponding reaction with 1 : 2-dimethoxybenzene, veratrole, was investigated in order to assess the feasibility of preparing tropolones in this manner. The ring expansion of benzenoid compounds with diazomethane and its derivatives is well known, the reaction having been studied in detail by Buchner and his co-workers (*Ber.*, 1920, **53**, 865, and numerous earlier papers). They showed that the first-formed norcaradienecarboxylic esters (V) can be rearranged, especially by alkalis or at high temperatures, to derivatives of phenylacetic acid or the *cyclo-*

heptatrienecarboxylic acids (*e.g.*, VI). Other workers (*e.g.*, Smith *et al.*, *J. Amer. Chem. Soc.*, 1934, **56**, 2167; 1938, **60**, 648; Drake and Sweeney, *J. Org. Chem.*, 1946, **11**, 67) have extended Buchner's observations, and in particular, Plattner and his school have used the method for



the synthesis of the azulene ring system from indanes (review: Pommer, *Angew. Chem.*, 1950, **62**, 281). In the case of veratrole, the dimethoxy*cyclo*heptatrienecarboxylic ester formed as one of the products from the diazoacetic ester condensation is an enol ether of a dihydrotropolonecarboxylic ester, and thus in order to obtain the tropolonecarboxylic ester itself it was necessary to effect an oxidative acid hydrolysis. Of the various reagents tried for this step, bromine (1 mol.) in chloroform solution proved to be the most effective, and a tropolonecarboxylic ester was thereby obtained in approximately 7% overall yield from veratrole. Smaller yields were also obtained by the use of *N*-bromosuccinimide in carbon tetrachloride solution, a reagent which we had already used in a preparation of benzotropolone from 3 : 4-benzo*cyclo*heptane-1 : 2-dione (cf. Cook and Somerville, *Nature*, 1949, **163**, 410). Tropolonecarboxylic ester was extracted from the crude reaction product by virtue of its solubility in sodium hydrogen carbonate solution, and was readily purified by crystallisation or by sublimation under reduced pressure. It formed beautiful yellow needles, which gave green colours with both ferric chloride and cupric sulphate, and like the other tropolones it gave no typical carbonyl reactions. The most convincing evidence for the presence of the tropolone ring system in the ester came from the infra-red spectrum, which showed bands at 1736 (carbethoxy-group) and at 1620, 1560, 1478, 1440, and 1250 cm.$^{-1}$, which are characteristic of the tropolone nucleus (Scott and Tarbell, *J. Amer. Chem. Soc.*, 1950, **72**, 240). Since this work was first reported in preliminary form (Bartels-Keith and Johnson, *Chem. and Ind.*, 1950, 677), there has been much further study of the infra-red spectra of tropolones (*e.g.*, Koch, *J.*, 1951, 512; Haworth and Hobson, *ibid.*, p. 561; Aulin-Erdtman, *Acta Chem. Scand.*, 1950, **4**, 1490; Johnson, Sheppard, and Todd, *J.*, 1951, 1139), so this ring system may now be detected readily by the characteristic infra-red spectrum. The ultra-violet absorption spectrum of the tropolonecarboxylic ester is shown in the figure.

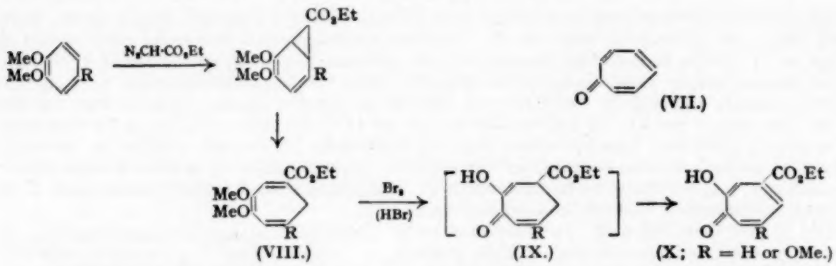
Ultra-violet absorption spectra of tropolone-β-carboxylic acid (———) and its ethyl ester (- - - - -) in 95% ethanol solution.



Since the initiation of the above synthesis, several other routes to the tropolones have been described, in particular the dehydrogenation of *cyclo*heptane-1 : 2-dione (Cook, Gibb, Raphael, and Somerville, *Chem. and Ind.*, 1950, 427; *J.*, 1951, 503) and the degradation of purpurogallin to β-methyltropolone and thence to tropolone itself (Haworth and Hobson, *loc. cit.*; *Chem. and Ind.*, 1950, 441). In another approach, Doering and Knox (*J. Amer. Chem. Soc.*, 1950, **72**, 2305; 1951, **73**, 828) have prepared tropolone by the permanganate oxidation of *cyclo*heptatriene (tropilidene), itself obtained either from *cyclo*heptene by dehydrogenation or from benzene and diazomethane by irradiation. It is obvious that Doering and Knox's method bears a close relation to the present method, but the presence of the oxygen atoms as protected hydroxy-groups in the nucleus before ring expansion avoids the permanganate oxidation which gives rise to many by-products. This has been recognised by the American workers, for in a recent note describing the preparation of the parent ketone, tropone (VII), they use the ring expansion of anisole with diazomethane in ultra-violet light. For studies of the mechanism of these ring-expansion reactions and the determination of the exact structure of the intermediates, the use of diazoacetic ester has the obvious advantage over diazomethane

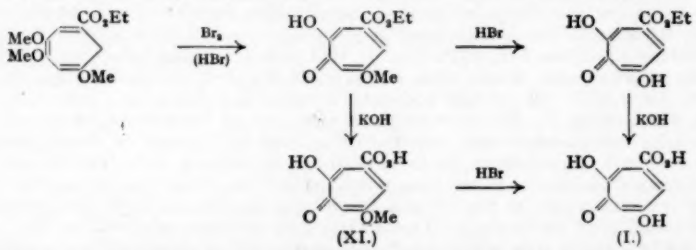
in that the carbon atom introduced by the reagent is marked by the carbethoxy-group. These studies, which should lead to improvements in the low yields of tropolones obtained at present, will be discussed in forthcoming papers.

Hydrolysis of the tropolonecarboxylic ester gave the corresponding acid, m. p. 217—219°, and by direct comparison it was shown to be identical with tropolone-β-carboxylic acid, an authentic specimen of which was kindly provided by Professor R. D. Haworth. As the decarboxylation of this acid to tropolone itself has already been described (Haworth and Hobson, *loc. cit.*), the present method represents a further synthesis of tropolone itself. The course of the veratrole–diazoacetic ester reaction to produce tropolone-β-carboxylic ester (X; R = H) may therefore be represented as follows (R = H), although the exact positions of the double bonds in (VIII) and (IX) have still to be determined.



Besides tropolone-β-carboxylic ester there was also obtained, in approximately equal quantities from the reaction of veratrole with diazoacetic ester, a colourless acid $C_8H_8O_4$, m. p. 149°, the structure of which is under consideration, but which is not a tropolone, on the basis of its infra-red spectrum.

The extension of this synthesis to the preparation of other naturally occurring tropolones, including stipitatic acid, is of course obvious. Thus the use of 1 : 2 : 3 : 4-tetramethoxybenzene and diazoacetic ester should give puberulic acid, and likewise the various alkyltropolones should be obtained from the alkylveratroles, or from veratrole itself with the diazoalkanes or diazoalkanecarboxylic esters. From the reaction of 1 : 2 : 4-trimethoxybenzene with diazoacetic ester, the crude trimethoxy*cyclo*heptatrienecarboxylic ester was isolated in 44% yield. Considerable insight into the nature of the impurities in this product has been gained from a study of the acids obtained by alkaline hydrolysis, but this aspect of the subject will be discussed in detail in a later paper. Bromine (1 mol.) in acetic acid proved to be the most satisfactory reagent for the oxidative hydrolysis and from the crude reaction mixture there were obtained three products, ethyl *O*-methylstipitatate (X; R = OMe), ethyl stipitatate, and an unidentified bright yellow compound, m. p. 182—184°, which is still under investigation. Synthetic stipitatic ester was identical with the ethyl ester prepared from authentic stipitatic acid. Alkaline hydrolysis of the two esters gave the corresponding acids, stipitatic acid and its *O*-methyl ether, the latter being identical with the product obtained by direct methylation of stipitatic acid with methyl sulphate (Birkinshaw, Chambers, and Raistrick, *loc. cit.*). The



formulation of *O*-methylstipitatic acid as (XI) rather than as the isomeric methyl ether is preferred because the compound is not easily hydrolysed (*e.g.*, it was recovered unchanged from a solution in 48% hydrobromic acid after 20 minutes at 50°), whereas tropolone methyl

ethers are known to be readily hydrolysed in acids and alkalis. This is in agreement with Dewar's suggestion (*loc. cit.*) concerning the nature of this compound. A second synthesis of stipitatic acid was achieved by the demethylation of this *O*-methyl ether with hydrogen bromide, and the ethyl stipitatate isolated from the products of the original bromination was no doubt formed by a similar mechanism. Synthetic stipitatic acid was identical with the natural product in all respects, including the infra-red spectrum (Johnson, Sheppard, and Todd, *loc. cit.*; Aulin-Erdtman, *loc. cit.*).

Experimental.

Reaction of Methoxybenzoquinone and Ethyl Diazoacetate.—Methoxybenzoquinone (3 g.; Erdtman, *Proc. Roy. Soc.*, 1933, *A*, **143**, 177) was dissolved in boiling light petroleum (400 c.c.; b. p. 80—100°), and ethyl diazoacetate (2·4 g.) added slowly during 1 hour. The reaction mixture was heated under reflux for a further hour, during which some darkening took place. On cooling, a brown solid was precipitated which was separated and washed with acetone (3 c.c.), leaving a greenish-brown crystalline solid (940 mg.), m. p. 127—132°. Unchanged methoxybenzoquinone was removed from the product by sublimation at 100°/1 mm., and the brownish residue (150 mg.) was crystallised from ethanol to give pale yellow needles of *ethyl 5(6)-methoxyindazole-4 : 7-quinone-3-carboxylate*, m. p. 245—247° (decomp.) (Found : C, 53·1; H, 3·8; N, 11·3. $C_{11}H_{10}O_5N_2$ requires C, 52·8; H, 4·0; N, 11·2%).

Reaction of Veratrole and Ethyl Diazoacetate.—Redistilled veratrole (200 g.) was heated to 150°, and ethyl diazoacetate (40 g.) added dropwise with stirring during 2 hours. After 15—30 minutes a steady stream of nitrogen was evolved, and the temperature was kept at 150—155° throughout the addition. The mixture was kept at the same temperature for a further 3½ hours, after which the volume of nitrogen evolved (collected over water) was 92% of theory (calc. on diazoacetic ester). The dark red product was distilled under reduced pressure to remove the excess of veratrole (187 g.; b. p. 50—60°/0·2 mm.), and the residue distilled in high vacuum, yielding a yellow oil (17·8 g.; b. p. 80—95°/2 × 10^{-4} mm.). The distillate was redistilled, and a middle cut (b. p. 84—87°/1·3 × 10^{-4} mm., n_D^{15} 1·5175) taken for analysis (Found : C, 63·7; H, 7·1. $C_{18}H_{14}O_4$ requires C, 64·3; H, 7·1%). The *ethyl dimethoxycyclo-heptatrienecarboxylate* slowly darkened on exposure to air, and formed deep yellow solutions in concentrated sulphuric acid or hydrochloric acid. Permanganate in acid or alkaline solution was instantly reduced and bromine was immediately decolorised. The ultra-violet absorption spectrum of a solution in 95% ethanol showed a maximum at 2740—2750 A. (log $\varepsilon_{max.}$ 3·51), with inflexions at 3120—3130, 3060—3100, 2950—2970 A.; log $\varepsilon_{max.}$ 3·30, 3·30, and 3·33, respectively.

Ethyl Tropolone-β-carboxylate.—The foregoing crude ester (1·84 g.) was dissolved in chloroform (30 c.c.), and a solution of bromine (1·35 g., 1 mol.) in chloroform added. Hydrogen bromide was evolved, and the mixture was then heated for 5 hours under reflux. The chloroform was removed, the residue dissolved in ether, and the solution repeatedly extracted with saturated sodium hydrogen carbonate solution (8 × 20 c.c.). The combined yellow aqueous extracts were acidified and the solution was extracted with ether (6 × 20 c.c.). The solvent was removed from the ethereal extracts, and the residue sublimed under reduced pressure (80—100°/20 mm.) to give *ethyl tropolone-β-carboxylate* as yellow needles (110 mg., 6·9%) which after recrystallisation from light petroleum (b. p. 40—60°) had m. p. 90—91° (Found : C, 61·2; H, 5·1. $C_{10}H_{10}O_4$ requires C, 61·3; H, 5·2%). The ultra-violet absorption spectrum of a solution in 95% ethanol showed maxima at 2505, 3130, 3250, 3745, and 3885 A.; log $\varepsilon_{max.}$ 4·43, 3·69, 3·73, 3·72, and 3·68, respectively.

When the crude product before sublimation was extracted with light petroleum (b. p. 40—60°) so as to remove most of the tropolone-β-carboxylic ester, a further extraction with light petroleum (b. p. 80—100°) yielded a second product (187 mg. from 15·2 g. of crude dimethoxy*cyclo*heptatrienecarboxylic ester); purified by sublimation at 100°/0·05 mm. and crystallisation from *n*-hexane, this *substance* formed colourless needles, m. p. 149° (decomp.) (Found : C, 57·8; H, 3·5. $C_8H_6O_4$ requires C, 57·8; H, 3·6%). The ultra-violet absorption spectrum (95% ethanol as solvent) showed maxima at 2100—2110, 2530, and 3170 A.; log $\varepsilon_{max.}$ 4·15, 4·44, and 3·48, respectively. Alkaline solutions were colourless and the acid gave no marked ferric test.

Tropolone-β-carboxylic Acid.—Tropolone-β-carboxylic ester (64 mg.) was dissolved in aqueous sodium carbonate (41 mg. in 5 c.c.), and the solution heated under reflux for 2½ hours. No change in colour was observed. The solution was cooled, acidified with 3N-hydrochloric acid, and set aside at 0°; a yellow solid (33 mg.) separated, and a further quantity (19 mg.) was obtained by extraction of the filtrate with ether (6 × 3 c.c.) and evaporation of the combined ethereal extracts. The crude product, m. p. 213—218°, was obtained as buff-coloured needles, m. p. 217—219°, not depressed on admixture with an authentic specimen, m. p. 217—218° (Haworth and Hobson, *J.*, 1950, 561) (Found : C, 57·8; H, 3·7. Calc. for $C_8H_6O_4$: C, 57·8; H, 3·6%). The ultra-violet absorption spectrum in 95% ethanol showed maxima at 2440—2460, 3240—3250, and 3670—3690 A.; log $\varepsilon_{max.}$ 4·49, 3·79, and 3·77, respectively. The infra-red spectrum of the acid, as a mull in Nujol, showed absorption maxima at 1770, 1605, 1530, 1468, 1427, 1400, 1272, 1245, 1220, 950, 790, and 707 $cm.^{-1}$. The acid gave a green colour with ferric chloride and formed yellow solutions in alkalis. Like the corresponding ester, the solution in concentrated hydrochloric acid was colourless.

1 : 2 : 4-*Trimethoxybenzene.*—Methoxyquinol (Dakin, *J. Amer. Chem. Soc.*, 1909, **42**, 496) was methylated with methyl sulphate to yield 1 : 2 : 4-trimethoxybenzene (60%; b. p. 96°/0·4 mm.).

Reaction of 1 : 2 : 4-*Trimethoxybenzene with Ethyl Diazoacetate: Ethyl* O-*Methylstipitatate.*—Ethyl diazoacetate (60 c.c.) was added dropwise during 2½ hours to 1 : 2 : 4-trimethoxybenzene (300 g.) at 150° with stirring, and the mixture kept at this temperature for a further 3½ hours; the unchanged trimethoxybenzene (250 g.) was then removed by distillation at 0·1 mm. The residues from two such

preparations were combined and distilled, and the following fractions were taken: (i) b. p. 55—70°/10^{-4} mm. (8·8 g.), giving a red colour with sulphuric acid; (ii) b. p. 80—100°/10^{-4} mm. (36·8 g.), red colour with sulphuric acid; (iii) b. p. 109—120°/10^{-4} mm. (29·0 g.), orange colour with sulphuric acid.

Fraction (ii) (35·8 g.) was oxidised in small portions (1·5 g.) each in acetic acid (20 c.c.) and water (4 c.c.). Bromine (0·3 c.c., 1 mol.) in acetic acid (10 c.c.) was added dropwise with shaking during 15 minutes, and the mixture set aside at room temperature for a further 15 minutes and then heated for 40 minutes on the steam-bath. The acetic acid was removed *in vacuo* and the residue extracted with N-sodium carbonate solution (3 × 15 c.c.). The extract was washed with ether (2 × 30 c.c.), acidified with 3N-hydrochloric acid, and extracted with ether (3 × 35 c.c.). The combined ethereal extracts were washed well with water and dried, and the solvent removed. The residue was sublimed at 100—110°/0·02 mm. and the combined sublimates were percolated for a short time with ether in order to remove a more soluble yellow compound (see below). The non-volatile fraction remaining after the sublimation was treated separately (see below). The portion of the sublimate insoluble in cold methanol was then extracted with boiling methanol (Soxhlet), and the extract on concentration yielded crude *ethyl O-methyl stipitatate*, which was purified by recrystallisation from the same solvent. The ether-ester (200 mg.) formed pale yellow needles, m. p. 154—156° (Found, in a sample dried at 60° for 3 hours in a vacuum: C, 58·4, 58·9; H, 5·4, 5·3. $C_{11}H_{12}O_5$ requires C, 58·9; H, 5·4%). The ultra-violet absorption spectrum in 95% methanol showed maxima at 2610 and 3500 (broad) A.; log $\varepsilon_{max.}$ 4·32 and 3·56, respectively.

The ether-soluble fraction of the crude sublimate was combined with the mother-liquors from the crystallisation of ethyl *O*-methylstipitatate and after fractional crystallisation from methanol, a *substance* was obtained which crystallised in bright yellow needles (30 mg.), m. p. 182—184° (slight decomp.) (Found, in a sample dried overnight at room temperature in a vacuum: C, 56·9; H, 5·1. $C_{10}H_{10}O_5$ requires C, 57·1; H, 4·8%). The ultra-violet absorption spectrum of an ethanol solution showed maxima at 2430, 3360, and 4040 A.; $E^{1\%}_{1\,cm.}$ 1230, 367, and 345, respectively.

The non-volatile residues from the sublimations were combined and triturated with cold methanol, and the insoluble residue crystallised from hot methanol (charcoal) to give pale yellow plates of *ethyl stipitatate* (40 mg.), m. p. 240° (decomp.), raised to 244° (decomp.) on recrystallisation from the same solvent and undepressed in admixture with a sample of the ester prepared from the natural acid (see below) (Found, in a sample dried overnight at room temperature in a vacuum: C, 57·1; H, 4·6. $C_{10}H_{10}O_5$ requires C, 57·1; H, 4·8%). The ultra-violet absorption spectrum of an ethanol solution showed maxima at 2640, 3640, and 3700 A.; log $\varepsilon_{max.}$ 4·48, 3·61, and 3·61, respectively.

O-Methylstipitatic Acid.—Hydrolysis of the ethyl ester with N/10-aqueous potassium hydroxide at 50° for 1 hour gave almost quantitative yields of *O*-methylstipitatic acid, crystallising from methanol in pale yellow flat needles, m. p. 262—264° (decomp.), alone or mixed with an authentic specimen prepared from stipitatic acid (Birkinshaw, Chambers, and Raistrick, *loc. cit.*) [Found, in a sample dried overnight at room temperature in a vacuum: C, 55·6; H, 4·0; OMe (Zeisel), 15·6. Calc. for $C_9H_8O_5$: C, 55·1; H, 4·1; 1OMe, 15·8%]. The ultra-violet absorption spectrum showed maxima at 2580, 3160, and 3560 A.; log $\varepsilon_{max.}$ 4·48, 3·53, and 3·61, respectively.

Stipitatic Acid.—(*a*) *O*-Methylstipitatic acid (40 mg.) in hydrobromic acid (1 c.c. of 48%) was heated at 110° for 12 hours. Stipitatic acid crystallised out from the cooled reaction mixture and was recrystallised from boiling water, forming small pale yellow plates (55 mg.), m. p. 280° (decomp. with previous darkening) alone and mixed with an authentic specimen. For analysis, it was sublimed at 180°/2 × 10^{-4} mm., the m. p. being thereby raised to 282° (Found: C, 53·0; H, 3·5. Calc. for $C_8H_6O_5$: C, 52·7; H, 3·3%).

(*b*) Hydrolysis of ethyl stipitatate with N/10-aqueous potassium hydroxide at 50° for 1 hour gave stipitatic acid, m. p. 282° (decomp.), identical with the product obtained in (*a*) (Found: C, 52·5; H, 3·6%). The ferric colours of stipitatic acid, its *O*-methyl ether, ethyl ester, and *O*-methyl ether ethyl ester, as determined with alcoholic solutions and excess of the reagent, were all green.

Ethyl Stipitatate.—Stipitatic acid (30 mg.) was heated under reflux with absolute ethanol (5 c.c.) containing concentrated sulphuric acid (3 drops) for 2 hours. After dilution and extraction with ether in the usual way, *ethyl stipitatate* was obtained as pale yellow plates, m. p. 244—246° (decomp.) (from methanol) (Found, in a sample dried overnight at 50° in a vacuum: C, 57·4; H, 5·0. $C_{10}H_{10}O_5$ requires C, 57·1; H, 4·8%).

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523. *Reaction-kinetic Investigations of the Incomplete Dissociation of Salts. Part III.* The Decomposition of Nitramide in Solutions of Some Metal Salts of Carboxylic Acids.*

By R. P. Bell and (Mrs.) G. M. Waind.

A kinetic study has been made of the decomposition of nitramide at 25° catalysed by the anions of five carboxylic acids, present in buffer solutions as the sodium, calcium, barium, or zinc salts. Values for the catalytic constants of the anions were calculated from the results for the sodium salts and closely obeyed the Brönsted relation. The reaction is specifically retarded by calcium, barium, and zinc ions. This retardation can be accounted for in terms of the partial formation of species such as $C_6H_5{\cdot}CH(OH){\cdot}CO_2Ca^+$ and $[O_2C{\cdot}CH_2{\cdot}CH(OH){\cdot}CO_2]Ca$. The values found for the dissociation constants of these species follow the same order as those previously obtained by other methods, but are uniformly somewhat higher. The discrepancy can be accounted for by assuming that the ions do not entirely lose their catalytic power when associated with a metallic cation, or by the equivalent assumption that the transition state in the reaction associates to some extent with these cations.

In the previous papers of this series (Bell and Prue, *J.*, 1949, 362; Bell and Waind, *J.*, 1950, 1979) it was shown that, when bivalent metallic cations exert specific negative salt effects in reactions involving hydroxyl ions, these effects can be accounted for quantitatively in terms of incomplete dissociation of the metallic hydroxides. The values of the dissociation constants of species such as $CaOH^+$ were in fair agreement with those obtained from other sources. The same cations were found to have no specific retarding effect in certain reactions, and this was interpreted in terms of the properties of the transition state. The present work extends this kinetic method of investigating incomplete dissociation by measurements of the rate of decomposition of nitramide, catalysed by the anions of acetic, mandelic, salicylic, malic, and fumaric acids in the presence of sodium, calcium, barium, and zinc ions.

Experimental.

Nitramide was prepared by the method of Marlies, LaMer, and Greenspan (*Inorg. Synth.*, **1**, 68, 193) as white leaflets and was stored in a stoppered bottle in a desiccator.

The reactions mixtures were prepared from buffer solutions of sodium hydroxide and acid, to which was added a solution of sodium chloride, calcium chloride, barium chloride, or zinc perchlorate. The ionic strengths of the final solutions were adjusted to approx. 0·1 or 0·2 by the addition of sodium chloride solution (except for the zinc salts, for which sodium perchlorate solution was used). The pH of the buffer solutions was kept below 5, so that the catalytic effect of the nitramide ions could be neglected.

The sodium hydroxide solution was free from carbon dioxide and was standardised against constant-boiling hydrochloric acid. Acetic acid was " AnalaR " glacial, and other acids were estimated to be 99·9% pure by titration. The solutions of sodium chloride and sodium perchlorate were prepared by weight from " AnalaR " materials. The calcium and barium solutions were prepared from $BaCl_2.2H_2O$ (" AnalaR ") and calcium chloride (" AnalaR dried "). They were standardised by gravimetric chloride determinations, and also by determining barium as sulphate and calcium as oxalate (permanganate titration). The zinc perchlorate solution was prepared by treating standard perchloric acid solution with an excess of zinc oxide. All solutions were made up with distilled water free from carbon dioxide.

The kinetic measurements were carried out with the apparatus described by Bell and Caldin (*Trans. Faraday Soc.*, 1951, **47**, 50: cf. also Bell and Trotman-Dickenson, *J.*, 1949, 1291). It had a total volume of about 20 c.c. and required only 4 c.c. of solution and 3 mg. of nitramide. The temperature was throughout 25° ± 0·01°. The reactions were always strictly of the first order, and velocity constants were calculated by Guggenheim's method (*Phil. Mag.*, 1926, **7**, 538).

Results.

Table I gives the data for the sodium salts of the monobasic acids, together with the catalytic constants of the anions (k_0). The last were obtained by deducting from the observed velocity constant k ($\log_{10}$, min.$^{-1}$) the water velocity k_w (taken throughout as 123×10^{-4}), and then dividing by the corrected anion concentration c. The latter was calculated from the stoicheiometric buffer composition and the

* Part II, *J.*, 1950, 1979.

thermodynamic dissociation constant of the acid, the hydroxyl-ion concentration being neglected. Activity coefficients were calculated from the equation given by Guggenheim (*Phil. Mag.*, 1935, **19**, 588):

$$-\log_{10} f_z = 0{\cdot}5z^2I^{\frac{1}{2}}/(1 + I^{\frac{1}{2}}) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where z is the valency of the ion and I the ionic strength. All concentrations are given in moles/l., and a and b denote respectively the stoicheiometric concentrations of acid and of sodium hydroxide. The solutions were made up to the stated ionic strength by the addition of sodium chloride.

TABLE I.

10^4a.	10^5b.	10^5c.	10^5k.	k_b.	I.	10^4a.	10^5b.	10^4c.	10^5k.	k_b.	I.
Sodium acetate.						Sodium mandelate.					
378	309	319	590	1·480	0·2	682	234	237	501	0·159	0·2
378 *	309	319	597	1·483	0·2	370	234	239	513	0·161	0·1
252	613	617	1036	1·481	0·2	732	467	470	860	0·157	0·2
252 *	613	617	1045	1·485	0·2	980	593	595	1020	0·154	0·1
			Mean	1·482					Mean	0·157	

10^4a.	10^4b.	10^4c.	10^5k.	k_b.	I.
Sodium salicylate.					
365	310	310	320	0·0657	0·2
553	496	496	460	0·0670	0·2
677	814	814	660	0·0660	0·2
				Mean 0·0663	

* These solutions contained, in addition, 0·0398M-$CaCl_2$.

Table II gives the results for the sodium salts of the dibasic acids. The catalytic constants of the singly charged anions (k_b') and of the doubly charged anions (k_b'') were obtained by combining the results of experiments in which the buffer ratio was varied over as wide a range as possible (subject to keeping pH less than 5). The true composition of each buffer solution was calculated by successive approximations from the following set of equations:

$$2[A^{--}] = b + [H^+] - [HA^-]$$
$$\log_{10}[H_2A] = \log_{10}[H^+] + \log_{10}[HA^-] - \log_{10}K_1 - f(I)$$
$$[HA^-] = a - [A^{--}] - [H_2A]$$
$$\log_{10}[H^+] = \log_{10}K_2 - \log_{10}[A^{--}] + \log_{10}[HA^-] + 2f(I)$$

in which a = total acid concentration (stoicheiometric), b = sodium hydroxide concentration (stoicheiometric), H_2A, HA^-, A^{--} = acid molecule, singly charged anion and doubly charged anion respectively, K_1, K_2 = first and second thermodynamic dissociation constants of the acid, and $f(I) = I^{\frac{1}{2}}/(1 + I^{\frac{1}{2}})$. The final results of the successive approximations are given in the Table. They should be related to the observed velocity by the equation $k = k_0 + k_b'[HA^-] + k_b''[A^{--}]$, and the best values for k_b' and k_b'' for a given acid were obtained by plotting $(k - k_0)/[HA^-]$ against $[A^{--}]/[HA^-]$. They are given before the results for each acid, and colum 4 of the table gives the velocity constants calculated from these values.

TABLE II.

10^4a.	10^4b.	10^4k, obs.	10^4k, calc.	$10^4[H_2A]$.	$10^4[HA^-]$.	$10^4[A^{--}]$.	$10^4[H^+]$.	I.
Sodium fumarate. $k_b' = 0{\cdot}113$, $k_b'' = 0{\cdot}687$.								
243	333	100	98	12·1	127	104	1·7	0·126
333	408	104	108	29·6	196	108	2·9	0·164
473	534	127	131	60·5	288	125	4·3	0·239
357	533	155	159	10·8	159	188	1·2	0·147
316	509	167	162	4·9	112	199	0·8	0·171
Sodium malate. $k_b' = 0{\cdot}270$, $k_b'' = 1{\cdot}75$.								
379	371	171	180	61·8	263	55·0	1·62	0·174
512	459	205	207	108·4	347	57·5	2·25	0·212
526 *	459	210	202	117·4	355	53·4	2·28	0·175
427	459	239	240	50·7	291	85·3	2·09	0·211
395 *	574	396	397	9·2	195	190	0·34	0·187
512	735	489	497	14·8	262	237	0·41	0·200

* In these solutions the ionic strength was adjusted with sodium perchlorate instead of with sodium chloride.

The experimental results for solutions containing bivalent metallic cations are given in Table III.

TABLE III.

10^4a.	10^4b.	$10^4[CaCl_2]$.	10^3k.	10^4a.	10^4b.	$10^4[BaCl_2]$.	10^3k.
	Calcium mandelate.				Barium mandelate.		
412	239	546	423	850	213 †	570	737
467	234	252	442	765	426	237	760
711	478	496	750	1081	536	298	900
1093	598	141	980				
397	111 *	—	445		Barium salicylate.		
				71	860	445	813
	Calcium salicylate.				Barium malate.		
451	408	506	290				
399	336	492	340	526	459	287	1950
653	598	425	510	351	459	287	2280
				395	574	718	3180
	Calcium malate.				Zinc malate.		
408	399	687	1360		$10^4[Zn(ClO_4)_2]$.		
526	495	532	1440				
427	459	266	1950	127	239	290	595
395	577	266	3110	288	232	89	710
512	735	372	3440	422	558	184	1851
				422	536	115	2200
	Calcium fumarate.				Zinc mandelate.		
318	343	443	791	788	223	184	372
473	534	665	1210	656	294	92	504
453	710	177	1990	985	357	69	626
473	763	532	2165	985	357	69	630

* $b = [Ca(OH)_2]$. † $b = [Ba(OH)_2]$.

DISCUSSION.

The third column of Table IV contains the catalytic constants derived from the experiments with sodium salts (cf. Tables I and II). The two figures in parentheses are from the measurements by Baughan and Bell (*Proc. Roy. Soc.*, 1937, *A*, **158**, 464); the agreement is excellent.

TABLE IV.

Anion.	K_{HB}.	k_b (obs.).	k_b (calc.).
Salicylate	$1{\cdot}06 \times 10^{-3}$	0·0662 (0·0665)	0·0663
Hydrogen fumarate	$9{\cdot}6 \times 10^{-4}$	0·113	0·120
Mandelate	$3{\cdot}88 \times 10^{-4}$	0·157	0·142
Hydrogen malate	$3{\cdot}5 \times 10^{-4}$	0·270	0·265
Fumarate	$4{\cdot}1 \times 10^{-5}$	0·687	0·924
Acetate	$1{\cdot}75 \times 10^{-5}$	1·48 (1·48)	1·49
Malate	$9{\cdot}0 \times 10^{-6}$	1·75	2·92

The last column of Table IV contains the values of k_b calculated from the expression

$$k_b/q = 3{\cdot}12 \times 10^{-4}\,(p/qK_{HB})^{0{\cdot}758}$$

where K_{HB} is the thermodynamic dissociation constant of the acid conjugate to the catalysing anion, p is the number of equivalent protons in this acid, and q the number of equivalent points in the anion at which a proton can be attached. This the expression which Baughan and Bell (*loc. cit.*) found to hold for eight singly charged anions, and it agrees well with experiment for the singly charged anions in Table IV. The two doubly charged anions give values of k_b somewhat lower than predicted by the above expressions: this agrees with the original findings by Brönsted and Pedersen at 15°, and is also in agreement with Pedersen's theoretical prediction about the effect of charge (Brönsted and Pedersen, *Z. physikal. Chem.*, 1923, **108**, 185; Pedersen, *J. Phys. Chem.*, 1934, **38**, 581).

Table I shows that the addition of calcium ions to an acetate solution has no effect on its catalytic power, in agreement with the view that calcium acetate is almost completely dissociated. On the other hand, all the values of k in Table III are smaller than those calculated from the stoicheiometric anion concentrations and the catalytic constants given in Table IV. The following procedure was adopted to find out whether this reduction could be consistently

interpreted in terms of the removal of a fraction of the anions by association with metallic cations.

In the experiments with monobasic acids (mandelic and salicylic) the concentration of free anions $[A^-]$ was taken as $(k - k_0)/k_b$, where k_b is the catalytic constant of the anion derived from experiments with sodium salts (cf. Table I). This is equivalent to assuming that the association product MA^+ exerts no catalytic effect. The value of $[A^-]$ was then combined with the stoicheiometric composition of the solution to give $[MA^+]$ and $[M^{++}]$, taking into account the change of $[H^+]$ produced by displacement of the buffer ratio. The concentration dissociation constant $K_c = [A^-][M^{++}]/[MA^+]$ was converted into the thermodynamic constant K_d by using activity coefficients given by equation (1).

In interpreting the results for malic acid it is necessary to take into account the two equilibria defined by $K_c' = [HA^-][M^{++}]/[MHA^+]$, $K_c'' = [A^{--}][M^{++}]/[MA]$, with the corresponding thermodynamic constants K_d' and K_d''. It is not possible to derive both these constants directly from the experimental results, and the procedure adopted was to select by trial and error the value of K_d' which gave the best constancy in K_d''. As a first approximation K_d' was put equal to K_d for the mandelate of the same cation, since there is evidence (Davies, *J.*, 1938, 277) that the anions of a number of different α-hydroxy-acids associate to about the same extent with a given cation. For a given value of K_d' it was then possible to calculate the composition of each solution and the corresponding value of K_d''. This was done by successive approximations from the following set of equations, in which s is the concentration of added salt [$CaCl_2$, $BaCl_2$, or $Zn(ClO_4)_2$], and the other symbols have been previously defined:

$$[A^{--}] = (k - k_0 - k_b'[HA^-])/k_b''$$
$$\log_{10}[H^+] = \log_{10} K_2 + \log_{10}[HA^-] - \log_{10}[A^{--}] + 2f(I)$$
$$\log_{10}[H_2A] = \log_{10}[H^+] + \log_{10}[HA^-] - \log_{10} K_1 - f(I)$$
$$\log_{10}[MHA^+] = \log_{10}[M^{++}] + \log_{10}[HA^-] - \log_{10} K_d' + 2f(I)$$
$$[MHA^+] + 2[M^{++}] = [HA^-] + 2s + 2[A^{--}] - b - [H^+]$$
$$[MA] = s - [MHA^+] - [M^{++}]$$
$$[HA^-] = a - [MHA^+] - [MA] - [A^{--}] - [H_2A]$$
$$K_c'' = [M^{++}][A^{--}]/[MA]$$
$$\log_{10} K_d'' = \log_{10} K_c'' - 4f(I)$$

The values finally obtained for the malates are given in Table V. A large proportion of the observed catalysis is due to the doubly charged anion, and the effect of added cations is chiefly to remove this ion. For this reason the values of K_d' are uncertain, but the values of K_d'' are little affected by this uncertainty. The same method of treatment is applicable in principle to the results for fumaric acid, but here the association with cations is much smaller and the observed velocities can be accounted for by assuming that only one type of association, $M^{++} + A^{--} \rightleftharpoons MA$, is present. The corresponding values of K_d'' are given in Table V.

The mean values of the dissociation constants obtained are collected in Table VI. Our results can be compared with data from two other sources. Cannan and Kibrick (*J. Amer. Chem. Soc.*, 1938, **60**, 2314) give concentration dissociation constants for the metallic salts of many organic acids, including malic acid. These were obtained by observing the change of pH produced when salt solutions (*e.g.*, $CaCl_2$, $BaCl_2$, or $ZnCl_2$) are added to dilute buffer solutions at an ionic strength of about 0·2. Davies (*J.*, 1938, 276; 1939, 349; and other papers) has given thermodynamic dissociation constants for several of the salts we have studied, derived from solubility measurements at ionic strengths below 0·07. The values of K_d depend to some extent on the equation employed for ionic activity coefficients. To facilitate comparison with Davies's values we have recalculated K_d values from the K_c values in the present paper and from those given by Cannan and Kibrick using Davies's expression

$$-\log_{10} f_z = 0{\cdot}5z^2\left\{\frac{I^{\frac{1}{2}}}{1 + I^{\frac{1}{2}}} - 0{\cdot}2I\right\} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

in place of equation (1). The comparison of the different values is shown in Table VII. This shows clearly that all the values obtained by the kinetic method are higher than those obtained by other methods. Incorrect allowance for ionic activity coefficients might account for the difference between values derived from measurements at $I \sim 0{\cdot}2$ and those of Davies, but

TABLE V.

(The order of experiments is the same as in Table III.)

10^4[A$^-$].	10^4[CaA$^+$].	10^4[Ca^{++}].	I.	K_c.	K_d.
Calcium mandelate.					
191	54	492	0·178	0·173	0·045
203	35	218	0·091	0·128	0·043
399	83	413	0·200	0·199	0·048
556	48	93	0·089	0·117	0·041
211	15	96	0·097	0·138	0·046
				Mean	0·044
Calcium salicylate.					
399	9·7	496	0·200	1·63	0·39
327	9·1	483	0·200	1·41	0·34
587	11·1	414	0·200	1·61	0·38
				Mean	0·37

10^4[HA$^-$].	10^4[A^{--}].	10^4[Ca^{++}].	10^4[CaA].	10^4[CaHA$^+$].	I.	K_c''.	K_d''.
Calcium malate (K_d' = 0·060).							
221	37	608	29	50	0·232	0·078	0·0040
282	32	436	21	75	0·186	0·067	0·0042
263	64	218	22	26	2·160	0·063	0·0045
173	144	200	50	17	0·133	0·058	0·0049
230	154	260	86	26	0·170	0·047	0·0032
						Mean	0·0042
Calcium fumarate.							
193	66	431	12	—	0·171	0·24	0·016
280	112	648	17	—	0·259	0·42	0·019
193	240	159	19	—	0·142	0·20	0·016
164	270	502	30	—	0·254	0·45	0·021
						Mean	0·018

10^4[A$^-$].	10^4[BaA$^+$].	10^4[Ba^{++}].			I.	K_c.	K_d.
Barium mandelate.							
387	40	743	—	—	0·198	0·36	0·25
404	20	216	—	—	0·099	0·40	0·15
506	30	269	—	—	0·101	0·40	0·16
						Mean	0·20
Barium salicylate.							
845	15	430	—	—	0·200	2·3	0·55

10^4[HA$^-$].	10^4[A^{--}].	10^4[Ba^{++}].	10^4[BaA].	10^4[BaHA$^+$].	I.	K_c''.	K_d''.
Barium malate (K_d' = 0·17).							
338	52	272	4·6	11	0·162	0·31	0·014
192	114	250	13	14	0·223	0·22	0·011
177	147	659	44	14	0·271	0·22	0·009
						Mean	0·011

10^4[A$^-$].	10^4[ZnA$^+$].	10^4[Zn^{++}].	I.	K_c.	K_d.
Zinc mandelate.					
159	87	98	0·078	0·018	0·0066
243	60	32	0·098	0·013	0·0041
320	48	21	0·137	0·014	0·0041
323	49	21	0·177	0·014	0·0035
				Mean	0·0045

10^4[HA$^-$].	10^4[A^{--}].	10^4[Zn^{++}].	10^4[ZnA].	10^4[ZnHA$^+$].	I.	K_c''.	K_d''.
Zinc malate (K_d' = 0·0060).							
150	10	39	17	33	0·104	0·0025	0·00027
7	26	197	87	6	0·136	0·0058	0·00051
59	33	60	38	25	0·147	0·0052	0·00041
192	69	49	94	41	0·171	0·0036	0·00025
222	85	29	60	26	0·198	0·0041	0·00024
						Mean	0·00034

cannot explain discrepancies between measurements at comparable ionic strengths. Scatchard and Teft's data (*J. Amer. Chem. Soc.*, 1930, **52**, 2272) show that the zinc chloride solutions used by Cannan and Kibrick contained significant concentrations of $ZnCl^+$, which would cause their constant for zinc malate to be too low. However, this explanation does not apply for the calcium and barium salts.

It seems likely, therefore, that in the present reaction the decrease of velocity caused by the addition of bivalent cations is somewhat smaller than the decrease of anion concentration due to association. This state of affairs will arise if the metallic cations associate to some extent with the transition state of the reaction, though to a smaller degree than with the free anions. An alternative way of expressing the same fact is to state that the associated anions possess a catalytic power which is appreciable, though smaller than that of the free anions. Previous papers in this series have revealed two extreme types of behaviour in the effect of

TABLE VI.

Collected values.

	K_d'.			K_d'.		
	Ca.	Ba.	Zn.	Ca.	Ba.	Zn.
Mandelates	0·044	0·20	0·0045	—	—	—
Salicylates	0·37	0·55	—	—	—	—
Malates	0·06	0·17	0·006	0·0042	0·011	0·00034
Fumarates	—	—	—	0·018	—	—

TABLE VII.

C = Cannan and Kibrick's values (recalculated).
D = Davies's values.
K = Kinetic values, recalculated by using equation (2).

		Calcium.			Barium.			Zinc.		
		C.	D.	K.	C.	D.	K.	C.	D.	K.
Mandelates	K_d	—	0·035	0·051	—	0·17	0·20	—	—	0·0055
Salicylates	K_d	—	0·26	0·44	—	—	0·62	—	—	—
Acetates	K_d	0·6	1	>2	—	—	—	—	—	—
Malates	K_d'	0·03	—	0·08	0·14	—	0·22	0·011	—	0·010
	K_d''	0·0013	0·0022	0·0058	0·0030	0·0064	0·015	0·00020	—	0·00048
Fumarates	K_d''	—	0·010	0·025	—	—	—	—	—	—

association on the reactivity of the hydroxyl ion. In the first (found in the decomposition of diacetone alcohol and in the hydrolysis of the carbethoxytrimethylammonium ion) the reactivity is entirely removed by association with metallic cations, indicating that these cations do not associate appreciably with the transition state. In the second (the alkaline hydrolysis of ethyl acetate) the association of the hydroxyl ion with the same metallic cations produced no decrease in reaction velocity, showing that the association persists in the transition state. The behaviour reported in the present paper is intermediate between these two extremes.

Apart from the overall discrepancy discussed above, the values of the dissociation constants of salts obtained from these kinetic measurements follow the same trend as those obtained by other methods. For each acid the zinc salts are much weaker than the calcium salts, and the calcium salts weaker than the barium salts, while for a given cation the dissociation constants follow the series: acetate > salicylate > mandelate; fumarate > malate.

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524. *The Vapour Pressures of Concentrated Aqueous Sucrose Solutions up to the Pressure of 760 mm.*

By W. J. Dunning, H. C. Evans, and M. Taylor.

A method of determining the vapour pressure of pure sucrose solutions at relatively high temperatures is described. The design of the apparatus affords accurate control of the weight composition of the solution throughout the measurements and avoids errors inherent in earlier methods.

Systematic results covering a wide range of concentration and temperature are recorded. An empirical equation, relating vapour pressure to temperature, and enabling the elevation of the boiling point to be calculated, has been derived.

Although various investigators (Robinson and Sinclair, *J. Phys. Chem.*, 1933, **37**, 495; *J. Amer. Chem. Soc.*, 1934, **56**, 1830; Scatchard, Hamer, and Wood, *ibid.*, 1938, **60**, 3061) have used the isopiestic method for the determination of the activities of sucrose solutions at relatively low temperatures, the method has not been applied at higher temperatures. Attempts to determine systematically and accurately the vapour pressures of concentrated solutions at higher temperatures appear to be restricted to the work of Spengler, Böttger, and Werner (*Z. Wirtsch. Zuckerind.*, 1938, **88**, 521). Neither the work of Perman and Saunders (*Trans. Faraday Soc.*, 1923, **19**, 112) nor that of Downs and Perman (*ibid.*, 1927, **23**, 95) covers concentrations higher than 76%, *i.e.*, 76 g. of sucrose in 100 g. of solution, nor does either supply data for any one solution over a comprehensive range of temperature. Spengler's interest is centred mainly on the technically important question of the relation between the elevation of the boiling point and the pressure as read on the manometer. He appears not to have made adequate allowance for the superheating and other sources of error in the modified Beckmann method employed by him. Notwithstanding this, his experiments give results which, in general, appear to be concordant among themselves.

We have endeavoured to determine the vapour pressures of sucrose solutions at various temperatures and concentrations by a method designed to be capable, in favourable conditions, of rigid measurement of these quantities. This method depends on the attainment of a true static equilibrium.

Experimental.

In the course of this work, we have several times had occasion to modify the apparatus. The present form is shown in Fig. 1. It consists essentially of a flask *A* containing the solution to be investigated, connected with one limb of the manometer *B*, the other limb of which is connected with the barometer. The pressure in the barometer can be adjusted to be equal to the vapour pressure in flask *A* by admitting or withdrawing air through the two-way tap *M* until the mercury is at the same level in the two arms of the manometer. To give freedom of movement, the manometer is connected through the train of tubes to the barometer by means of thick, water-jacketed pressure tubing, and is immersed in a rocking thermostat *C*. This is provided with a glass window *W* and is kept at a temperature about 1° higher than that of the main thermostat. By means of the air reservoir *L* under adjustable mercury pressure, the apparatus is adapted for use at pressures somewhat higher than atmospheric. The arrangement shown in Fig. 1*a*, consisting of a strong metal T-piece working in a glass socket, permits of free rotation of the tap attached to cork *D*, while the springs prevent leakage under excess internal pressure. For temperatures up to about 90°, the liquid used in the thermostats is water, covered, in the main thermostat, by crude heavy paraffin. For higher temperatures, dry crude paraffin is used in the main thermostat and refined paraffin in the rocking thermostat.

The Precision Apparatus.—The working thermometer, graduated in 0·1°, is calibrated at intervals of 5° against a National Physical Laboratory standard of which the limit of accuracy is ±0·02°. Besides being independently calibrated in the neighbourhood of 100° against the b. p. of water at known atmospheric pressure, its readings have been checked in the vapour-pressure apparatus by the determination of the vapour pressure of water at intervals of 5° from 60° upwards. The Reichsanstalt values for the temperatures corresponding to the different vapour pressures are taken as correct. Each thermometer reading in the apparatus is corrected for exposed stem. The barometer is enclosed in a water jacket, the temperature of which is read immediately after the pressure. Both the barometer and manometer readings are corrected for pressure towards the centres of curvature of the mercury menisci. A cathetometer is used for the reading of the mercury levels. The barometer readings are corrected for temperature and also for the effect of latitude on gravity. The glass barometer scale has been compared with a Zeiss scale over lengths of 100 mm. and the average divergence is found to be 0·07 mm.

In the earlier experiments, owing to the slight up and down temperature swing of 0·03—0·08°, causing a corresponding swing in the mercury levels in the manometer, the temperature was read at the instant that the mercury became level in the arms of the manometer. This necessitated two operators. In the later observations at higher temperatures, giving pressures approximately atmospheric, the use of an improved thermoregulator containing chlorobenzene greatly reduces this temperature variation and enables one operator (H. C. E.) to carry on the work. Increased accuracy is also attained by the use of a short-range N.P.L. calibrated thermometer, graduated in 0·05° and especially designed to afford satisfactory immersion in the thermostat.

Outline of Method.—Tate and Lyle's purest sucrose containing on a dry basis 99·99% of sucrose and 0·001—0·002% of invert sugar is used throughout. After very fine grinding to accelerate dissolution, the sucrose is dried at 60° under 5 cm. pressure in a stream of dry air. It is weighed into flask *A*, which

FIG. 1.

Apparatus for determination of the vapour pressure of solutions up to atmospheric pressure.

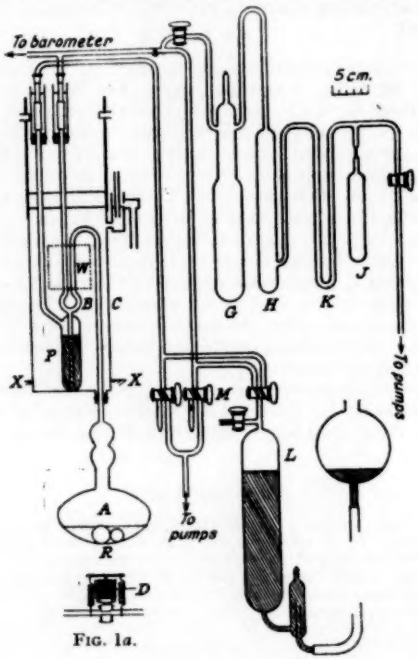


FIG. 1*a*.

XX = Water level in thermostat.

is then sealed on to the manometer, and the requisite quantity of water is transferred from a weight burette into tube *G*, the whole of the mercury in the manometer being in reservoir *P*.

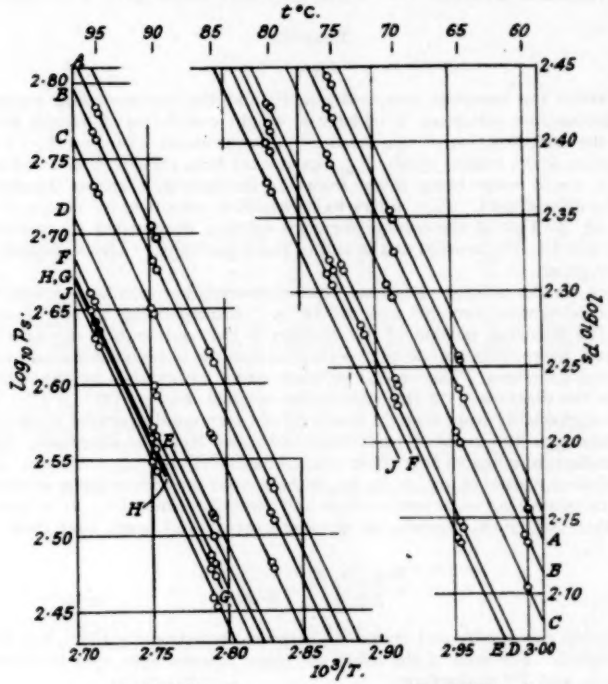
After tube *G* has been sealed, the water is immediately frozen by means of liquid air, and the apparatus exhausted by a Cenco Hyvac and a mercury-vapour pump, till no air can be detected by the McLeod gauge. During the pumping out of the air, tube *H* must be immersed in liquid air to detect and prevent any loss of water.

After complete evacuation, the mercury is pushed up into the manometer, thus isolating flask *A* from the water tubes. By repeated distillation from tube *G* to tube *H* and *vice versa*, and subsequent exhaustion when the water is frozen, the air can be removed from the water, which should be as free as possible from carbon dioxide before being put into the apparatus. Conductivity water is used, but as a final precaution, a few c.c. of water may be distilled over into tube *J*, which is then sealed off, and the water discarded after its weight has been determined. The mercury in the manometer is drawn down, the flask *A* immersed in carbon dioxide and methylated spirit, and the water is distilled into the sucrose. The flask is again closed by pushing the mercury up into the manometer, and the sugar is dissolved by rocking the thermostat *C*. With solutions of high concentration, it is preferable to obtain the requisite high temperature for solution by rapidly heating the flask in a large beaker, containing,

e.g., calcium chloride solution, and then replacing the beaker by the heated main thermostat. The thermostat *C* is continually rocked until equilibrium is attained, effective disturbance of the solution being ensured by the marbles *R*, which project through the surface. The mercury in the manometer is levelled, and the shaking stopped only for the time required to take the temperature reading. At least ten such readings are rapidly taken and the mean is the equilibrium temperature. The variation in the mean reading for duplicate sets of observations at any one temperature is not more than 0·02°. Any drift in the readings is taken to indicate that equilibrium has not been attained, and the process is repeated after further shaking.

FIG. 2.

Graphic relationship between $\log_{10} p_s$ *and* $10^3/T$.



A, 44·85% *soln.*
◒ A′, 44·87 ,,
B, 58·90 ,,
C, 69·91 ,,
D, 78·39 ,,
E, 79·97% *soln.*
F, 81·10 ,,
G, 82·05 ,,
H, 82·22 ,,
J, 83·22 ,,

◒ *With the exception of this point, the values of* A′ *coincide too closely with those of* A *to permit insertion in the graph.*

As an additional precaution, at least two independent readings are taken at each round temperature, and in the one case equilibrium is attained by lowering, and in the other by raising the temperature, thus lessening the possibility of unrecognised errors caused by the presence of condensed drops of solvent or of surface layers differing in concentration from the main liquid. Since inversion of sucrose is known to occur in aqueous solution at a rate depending on the temperature as well as on other factors, the readings are taken at temperatures which are not consecutive. Appreciable and gradually increasing inversion would, therefore, produce irregularities in the pressure–temperature curve, rather than a steady drift which might escape observation.

At the completion of the vapour-pressure measurements with any one solution, the total weight is redetermined to ensure that no loss of water has occurred. Any change in weight has, in every case, been found to be negligible, being of the order of 0·01% of the total weight of the solution. In calculating the coucentration of the solutions no correction for buoyancy of air is applied. The weight of water forming the vapour phase is subtracted from the total weight of water. This correction is

equivalent to an addition of 0·03—0·07 to the percentage of sucrose in solution, depending on the temperature, pressure, and dimensions of the apparatus, as well as on the concentration and total volume of the solution. At the end of every series of experiments with any one solution, the latter is examined by the polarimeter to determine the percentage of sucrose inverted. Also, in cases in which the solution has been subjected to higher temperatures, such as those reached in the determination of vapour pressures of the order of one atmosphere, the percentage of invert present is determined by Lane and Eynon's method to detect possible further breakdown of invert sugar. In some such cases, Clerget's method has also been used.

The experimental work falls into two parts. The first deals with solutions examined over a wide range of pressure, at temperatures in no case exceeding 95°; the second part is mainly concerned with the temperatures required to produce a vapour pressure of 760 mm., *i.e.*, with the elevation of the b. p. under normal atmospheric pressure.

Results.

Part I.

Table I records the observed temperatures, $t°$, and the corresponding vapour pressures, p_s (in mm.), for sucrose solutions, in which the weight concentration ranges from about 45 to 83%, and the mole-fraction of the sucrose, N_2, from about 0·04 to 0·20. The table also gives the elevation of the boiling points, $E_{exp.}$, calculated from these pressures and temperatures, the requisite b. p.s of water being taken from the Reichsanstalt Tables (Landolt–Börnstein, 1931, Erg. IIb, 1329 *et seq.*). $E_{calc.}$ refers to values of E smoothed by means of equation (2) (see below). At the foot of the column for each solution is recorded the percentage of the initial sucrose found to be inverted at the end of the experiment. These symbols are retained throughout the paper.

Fig. 2 shows, for the different solutions, the relationship between $\log_{10} p_s$ and $10^3/T$, where T is the absolute temperature (0° C = 273·16° K.), corresponding to the observed vapour pressure p_s. The left-hand portion of the diagram is the continuation upwards of the right-hand portion and, for reading, should be superimposed on the latter as indicated by the marginal temperatures and pressures. The vapour pressure curve for water is on the extreme right of each portion of the diagram. On the scale of the original drawing (21″ × 17″), these curves do not deviate appreciably from straight lines and are very nearly parallel to each other. The slopes of the lines give the heats of vaporization of water from the solutions. Any deviation from exact parallelism indicates that there is a heat of dilution.

For each solution, values of $\log_{10} p_s/p_w$ (p_w is the vapour pressure of water at the temperature T) have been calculated for each temperature and plotted against T^{-1}. It is quite clear from these curves that $\log_{10} p_s/p_w$ depends on the temperature and hence that there is a heat of dilution, $\Delta\bar{H}_1$, *i.e.*,

$$\frac{\partial \log_{10} p_s/p_w}{\partial(T^{-1})} = \frac{-\Delta\bar{H}_1}{2{\cdot}303\boldsymbol{R}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

In order to smooth the results and obtain an empirical equation for them, the following procedure was adopted. For each of the solutions, mean squares were used to obtain a relation between $\log p_s/p_w$ and T^{-1} of the form

$$\log p_s/p_w = A(N_2) + B(N_2)/T$$

where A and B are taken as independent of the temperature, but functions of N_2, the mole-fraction of the sucrose. Then the values of $\log p_s/p_w$ were calculated for the respective solutions at 80° (approximately the median temperature for all solutions). These values at 80° were smoothed by applying mean squares to find the constants a and b in the equation

$$\frac{1}{N_2}\left(\log_{10}\frac{p_w}{p_s}\right)_{80} - 0{\cdot}4343 = aN_2 + bN_2^2$$

The values of $B(N_2)$ were plotted against N_2 and it was found that, within the limits of the experimental accuracy, they were proportional to N_2^2. Finally, the results were assembled into an empirical relation

$$\log_{10} p_w/p_s = 0{\cdot}4343N_2 + (0{\cdot}4721 + 713/T)N_2^2 - 1{\cdot}32N_2^3 \quad . \quad . \quad . \quad . \quad (2)$$

which may be considered to represent our results for the vapour pressures of sucrose solutions from 45 to 85% concentration between the temperatures of 60° and 95°.

Calculations from this equation of p_s, for comparison with the experimental results, show close agreement. The degree to which it represents the experimental data may be judged from the values of $E_{calc.}$, the calculated elevation of the b. p., which are given in Table I.

TABLE I.

The vapour pressures of pure sucrose solutions at various temperatures.

N_2 = mole-fraction of sucrose; p_s = vapour pressure of the solution in mm. Hg at temperature $t°$ C; $E_{exp.}$ = elevation of b. p. calculated from p_s and $t°$ and the b. p. of water under pressure p_s: $E_{calc.}$ = elevation of b. p. calculated from equation (2).

	A				A′				B			
	g. of sucrose per 100 g. of solution.											
	44·85				44·87				58·90			
N_2	0·041043				0.041077				0·070135			
			E_s				E_s				E_s	
Temp. region	t, °C	p_s	exp.	calc.	t, °C	p_s	exp.	calc.	t,° C	p_s	exp.	calc.
60°	—	—	—	—	60·20	143·145	1·12	1·10$_6$	60·17	136·02	2·18	2·12
	—	—	—	—	—	—	—	—	60·40	137·63	—	—
65	65·09	179·38	1·08	—	64·89	177·75	1·08	—	64·88	169·37	2·14	—
	65·26	180·68	1·09	1·13$_3$	65·21	180·06	1·12	—	65·24	171·73	2·20	2·18
70	70·08	222·77	1·18	1·16$_5$	70·07	222·95	1·15	—	70·10	213·00	2·23	2·25
	70·39	226·16	1·14	—	70·46	227·05	1·12	—	70·21	213·57	2·27	—
75	74·98	274·70	1·19	1·20$_4$	74·98	274·18	1·24	—	74·89	260·86	2·32	—
	75·29	277·76	1·24	—	75·34	278·61	1·22	—	75·30	265·02	2·36	2·31
80	80·08	338·90	1·23	—	79·49	338·35	1·18	—	80·07	323·25	2·37	2·38
	80·29	341·61	1·24	1·24$_6$	80·21	340·90	1·22	—	80·36	327·18	2·37	—
85	84·97	412·38	1·24	—	84·73	407·99	1·27	—	84·77	389·45	2·47	2·47
	85·37	417·80	1·31	1·27$_6$	85·07	413·09	1·30	—	85·04	394·21	2·44	—
90	89·86	498·28	1·27	—	89·85	497·70	1·29	—	89·84	474·15	2·54	—
	90·28	505·56	1·31	1·32$_3$	90·27	505·67	1·29	—	90·18	480·69	2·54	2·53
95	94·89	601·29	1·32	1·36	94·47	592·83	1·28	—	95·10	578·59	2·56	2·60
	95·14	607·33	1·30	—	95·36	612·96	1·29	—	95·46	586·97	2·53	—
	1·17 *				2·21				1·07			

	C				D				E			
	g. of sucrose per 100 g. of solution.											
	69·91				78·39				79·74			
N_2	0·108957				0·160312				0·171601			
			E_s				E_s				E_s	
Temp. region	t, °C	p	exp.	calc.	t, °C	p_s	exp.	calc.	t, °C	p_s	exp.	calc.
60°	60·29	126·97	3·76	3·76$_1$	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—	—
65	65·20	158·30	3·87	3·88	65·00	139·22	6·51	6·57	65·16	135·86	7·20	7·23$_1$
	65·49	160·87	3·88	—	65·21	140·30	6·55	—	65·41	137·00	7·27	—
70	70·29	198·00	4·07	—	69·95	172·81	6·76	—	69·95	167·58	7·45	—
	70·70	201·79	4·05	4·00	70·07	174·37	6·69	6·75	70·20	169·58	7·43	7·43$_9$
									72·52	186·86	7·60	7·52$_4$
									72·76	189·49	7·53	—
75	75·10	243·12	4·18	—	74·07	206·34	6·92	6·90	74·91	207·47	7·64	—
	75·38	246·64	4·12	4·10	74·28	208·19	6·93	—	75·25	210·68	7·63	7·62$_7$
80	80·14	300·09	4·25	—	79·96	264·47	7·17	7·12	80·00	255·94	7·88	7·82$_4$
	80·33	302·03	4·29	4·23	80·27	267·16	7·13	—	80·19	258·92	7·80	—
85	85·14	366·54	4·36	4·33	85·10	324·65	7·30	7·31	85·14	315·57	8·03	8·02$_8$
	85·34	369·00	4·39	—	85·26	326·93	7·29	—	85·16	315·16	8·08	—
90	90·10	443·61	4·52	4·47	89·98	391·67	7·54	7·50	—	—	—	—
	90·39	448·65	4·52	—	90·14	395·28	7·47	—	—	—	—	—
95	94·99	532·44	4·66	—	—	—	—	—	—	—	—	—
	95·30	538·47	4·67	4·58	—	—	—	—	—	—	—	—
	0·68				0·36				See p. 2370.			

* Data in the last line of each section record the sucrose inverted as g. per 100 g. of original.

TABLE I—*continued.*

	F				G				J*			
	g. of sucrose per 100 g. of solution.											
	81·10				82·05				83·22			
N_2	0·184232				0·193924				0·20699			
			E,				E,				E,	
Temp. region	t, °c	p_s	exp.	calc.	t, °c	p_s	exp.	calc.	t, °c	p_s	exp.	calc.
75°	75·02	201·80	8·37	$8·41_3$	—	—	—	—	—	—	—	—
	75·43	205·32	8·39	—	—	—	—	—	—	—	—	—
80	80·05	248·24	8·68	$8·63_5$	—	—	—	—	79·95 [2]	232·96	10·02	10·10
	80·43	251·58	8·69	—	—	—	—	—	80·27 [2]	236·25	$10·01_5$	—
85	85·08	303·40	8·92	8·86	85·08	296·96	9·44	—	84·94 [2]	283·68	10·39	$10·41_8$
	85·40	306·74	8·98	—	85·45	300·31	9·54	$9·53_3$	85·27 [2]	287·17	10·43	—
90	90·06	368·42	9·15	$9·00_4$	90·12	360·52	9·75	$9·66_2$	89·96 [2]	347·32	10·51	$10·68_7$
	90·33	372·84	9·11	—	90·45	365·50	9·74	—	90·42 [2]	353·59	10·53	10·75
95	95·39	452·24	9·30	$9·34_5$	—	—	—	—	95·00 [1]	422·36	10·66	$10·95_8$
	95·73	457·37	9·34	—	95·71	446·31	9·98	10·05	95·37 [1]	427·17	10·75	—
	0·41				1·61				[1] 0·6, see p. 2370. [2] 1·01			

* For solution H, data are as follows: 82·22 g./100 g. of solution, N_2 = 0·193741, sucrose inverted 0·39.

t, °c	p_s	$E_{exp.}$	$E_{calc.}$	t, °c	p_s	$E_{exp.}$
95·00	432·10	10·09	$10·19_4$	95·35°	437·76	10·11

By means of this equation, the smoothed values of p_s, E, and $\log_{10} p_w/p_s$ have been calculated for 45—90% concentrations at 5% intervals and for 60—95° at 5° intervals. These values are listed in Table II.

TABLE II.

Values of $\log_{10}p_w/p_s$, p_s, *and* E, *calculated by means of equation* (2), *in which* p_w *and* p_s *represent the vapour pressure of water and of the solution, respectively, at the temperature* t_0, *and* E *represents the elevation of the boiling point at the same temperature.*

g. sucrose/ 100 g. soln.	45	60	65	70	75	80	85	90
$10^7 N_2$	412,839	731,698	890,396	1,093,737	1,363,621	1,739,111	2,297,273	3,214,257
t_0				$10^7 \times \log_{10} p_w/p_s$				
60°	222,887	452,454	584,477	770,222	1,044,493	1,475,920	2,216,244	3,656,379
65	222,348	450,761	581,969	766,436	1,038,609	1,466,350	2,199,546	3,623,690
70	221,813	449,116	579,533	762,761	1,032,907	1,457,058	2,183,333	3,591,949
75	221,300	447,571	577,165	759,192	1,027,348	1,448,033	2,167,585	3,561,121
80	220,821	445,966	574,876	755,723	1,021,957	1,439,264	2,152,283	3,531,167
85	220,340	444,457	572,634	752,352	1,016,716	1,430,757	2,137,409	3,502,047
90	219,774	442,990	570,417	749,073	1,011,620	1,422,450	2,122,946	3,473,731
95	219,419	441,563	568,347	745,883	1,006,662	1,414,386	2,108,873	3,446,183
				p_s, mm.				
60	141·907	134·601	130·570	125·104	117·447	106·349	89·674	64·366
65	178·180	169·051	164·020	157·199	147·649	133·801	113·016	81·419
70	220·636	210·740	204·506	196·057	184·232	167·091	141·360	102·203
75	274·738	260·790	253·123	242·733	228·199	207·131	175·505	127·332
80	337·496	320·445	311·074	298·386	280·644	254·932	216·332	157·483
85	412·154	391·421	380·037	364·631	343·098	311·899	265·063	193·591
90	499·816	474·766	461·048	442·466	416·510	378·916	322·473	236·273
95	602·668	572·674	556·143	533·866	502·753	457·701	390·062	286·683
				E°, c				
60	$1·10_5$	$2·23_3$	$2·88_9$	$3·78_1$	$5·10_9$	$7·16_4$	$10·62_4$	$17·12_3$
65	$1·14_0$	$2·30_3$	$2·96_7$	$3·89_3$	$5·25_2$	$7·36_0$	$10·90_6$	$17·55_4$
70	$1·17_2$	$2·36_9$	$3·05_1$	$4·00_4$	$5·39_6$	$7·55_9$	$11·18_8$	$17·97_7$
75	$1·21_4$	$2·43_7$	$3·13_4$	$4·11_7$	$5·54_6$	$7·76_1$	$11·47_5$	$18·40_6$
80	$1·25_0$	$2·51_8$	$3·24_0$	$4·24_4$	$5·70_5$	$7·97_6$	$11·77_1$	$18·85_4$
85	$1·28_4$	$2·58_9$	$3·31_8$	$4·34_5$	$5·85_0$	$8·17_7$	$12·06_2$	$19·28_9$
90	$1·32_8$	$2·66_9$	$3·42_5$	$4·48_4$	$6·01_8$	$8·39_2$	$12·36_4$	$19·74_2$
95	$1·36_6$	$2·73_4$	$3·51_2$	$4·59_7$	$6·17_8$	$8·61_7$	$12·66_8$	$20·20_1$

Also, by a method of approximations, equation (2) has been employed to calculate at the above regular intervals of concentration, the elevations of the b. p. under fixed pressures. Table III records these elevations for 760 mm. and the stated fractions of 760 mm. pressure.

TABLE III.

Elevations of the boiling point, E°, *calculated by means of equation* (2) *for sucrose solutions of concentration* C (*mole-fraction of sucrose* = N_2) *under pressure* p_s (*mm.*).

p_s, mm.		190	380	570	760
Concn. *C* (g. sucrose/100 g. soln.)	N_2	Elevation of boiling point, *E*°, under pressure p_s (mm.)			
45	0·0412839	1·15$_0$	1·27$_0$	1·35$_4$	1·41$_6$
60	0·0731698	2·33$_8$	2·57$_3$	2·73$_5$	2·85$_9$
65	0·0890396	3·02$_3$	3·31$_8$	3·52$_6$	3·68$_2$
70	0·1093737	3·99$_1$	4·36$_5$	4·63$_9$	4·84$_1$
75	0·1363621	5·41$_4$	5·92$_3$	6·28$_0$	6·54$_9$
80	0·1739111	7·68$_1$	8·39$_5$	8·86$_4$	9·23$_3$
85	0·2297273	11·58$_1$	12·62$_5$	13·30$_7$	13·84$_3$
90	0·3214257	19·24$_9$	20·88$_3$	21·97$_3$	22·81$_3$

Table III enables the calculated results to be compared with those of earlier investigators and with the results of the actual determinations in Tables I and V.

Further, with regard to the application of equation (2), it should be pointed out that the heat of dilution ΔH can be obtained from the coefficient of T^{-1}. This corresponds to the relation

$$\Delta\bar{H}_1 = -3{\cdot}3 \times 10^3 N_2^3 \text{ cals./mol.} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

and indicates that the heat of dilution is small. Direct measurements of the heat of dilution of sucrose have been made previously (Stackelberg, *Z. physikal. Chem.*, 1898, **26**, 533; Wood, *Trans. Faraday Soc.*, 1917, **13**, 123; Hunter, *ibid.*, 1926, **22**, 194; Vallender and Perman, *ibid.*, 1931, **27**, 126; Gucker, Pickard, and Planck, *J. Amer. Chem. Soc.*, 1939, **61**, 459), the results of Vallender and Perman being the most extensive. Although our relation (3) should be considered only as a term in an empirical expression for our vapour pressures, yet it is interesting to compare the value which Vallender and Perman obtained for the heat of dilution of 68·79% sucrose at 80° with the value obtained for (3). As regards temperature and concentration, this lies close to the median of our ranges of concentration and temperature. For a 68·79% solution ($N_2 = 0{\cdot}10394$) equation (3) gives −1·98 cals./g.; direct determination by Vallender and Perman gave −2·02 cals./g. This close agreement must be regarded as merely fortuitous.

Inversion.—The sucrose used throughout these investigations contains, initially, not more than 0·002% of invert sugar. During use in the vapour-pressure apparatus, this proportion increased to not more than 2·21% of the original weight of sucrose (see Table I). The extent of inversion depends on many factors, such as concentration, temperature, pH, and time of heating, and, in the case of pure sucrose solutions, it may be complicated by the presence of an induction period. The detailed investigation of inversion is outside the scope of the present work, but the following experiments provide some information on the condition of the solutions at various times during the consecutive vapour-pressure measurements.

A 44·92% solution, made up with water of conductivity 1×10^{-6} mho, is heated *in vacuo* for successive periods of 2 hours at 60°, 65°, 70°, 75°, and 80°. In the course of the experiment, the solution is left to cool overnight several times. The polarimeter reading is the same at the end as at the beginning, indicating that no change in concentration and no inversion measurable by this method has taken place. However, after subsequent consecutive two-hour periods of heating at 90°, 95°, and 100°, the percentages of sucrose inverted are 0·32, 0·76, and 3·0, respectively. After further heating for 13 hours at 100°, 99% of the sucrose present is found to be inverted.

In the case of solution " E," after the vapour pressure readings given in Table I had been taken up to 85°, the solution was kept at about 85° for a further 30 hours. After this prolonged heating, in all about 50 hours, vapour-pressure readings were taken at intervals of 1—2 hours, as shown in Table IV.

Owing to this inversion, N_1, the mole-fraction of the water in the solution, is reduced from 0·8284 to 0·8075, and N_2, the mole-fraction of the solute (sucrose and invert sugar) is increased to 0·1925. By calculation from equation (2), it is found that at this mole-fraction, an unin-

TABLE IV.

Solution E. Concentration: 79·74 g. of sucrose per 100 g. of solution: $N_2 = 0{\cdot}171610$.
h = total hours of heating before the readings are taken.
$t_0°$ = temperature of the solution under vapour pressure p_s.
$E_{exp.}$ = observed elevation of b. p.
$E_{calc.}$ = elevation calculated from equation (2), inversion being assumed not to have occurred.

h	t_0	p_s	$E_{exp.}$	$E_{calc.}$
54	82·40°	274·44	8·64	7·925
53	82·58	276·64	8·63	
48	87·46	339·33	8·58	8·28
49	87·64	340·98	8·64	
51	92·40	408·89	8·89	8·50
50	92·82	416·12	8·87	

Percentage of initial sucrose inverted after 54 hours = 12·2.

verted sucrose solution would show elevations of 9·39°, 9·54°, and 9·85° respectively at the mean temperature of each pair of readings. The elevations $E_{exp.}$ found for the partly inverted solute (Table IV, col. 4) are considerably less, indicating that the osmotic properties of sucrose solutions containing some invert sugar are not simply a function of the mole-fraction of the water, but depend also on the sizes of the molecules present. In the present case, it is fortunate that the invert sugar has not such a marked effect on the osmotic properties as one might expect from the reduction produced in the water mole-fraction, and we may assume that the vapour pressures recorded in Table I are not appreciably affected by the small amounts of inversion which have occurred. Nevertheless, in all work on sucrose solutions, it is obviously important to keep a strict check on inversion, to reduce as much as possible the time of exposure to high temperature, and to ensure the purity of the water. There is, however, some indication that the presence of even a small percentage of invert sugar tends to prevent the crystallisation of supersaturated solutions, thereby facilitating the determination of their vapour pressures. For example, several attempts to obtain an approximately 83% solution which was stable enough to give satisfactory vapour-pressure readings below 95°, were unsuccessful. In each of these cases, the times of heating (at 105—108°) to effect solution had been curtailed as much as possible, and the final inversion was found to be less than 1% (see Table I, solution J, concentration 83·22%, final inversion, 0·6% of the sucrose). Solution J_2, of appreciably the same composition as J_1, was then prepared by maintaining the high temperature of solution for a considerably longer period. By this means, consistent readings were obtained at 5° intervals to the 80° region. The solubility of sucrose at 80° is 78·805 g. per 100 g. of solution (Taylor, *J.*, 1947, 1682). The final inversion was, in this case, found to be 1·01% of the total sucrose.

Since temperature is one of the more important factors influencing the rate of inversion, the vapour-pressure determinations in the foregoing experiments have been limited to temperatures not exceeding 95°. In concentrated solutions, temperatures considerably higher than 95° are required to produce a vapour pressure of 760 mm. The determination of these higher temperatures (*i.e.*, the b. p.s of concentrated sucrose solutions under 760 mm.) by the vapour-pressure method has been carried out independently by one of us (H. C. E). The experimental work and results are described in Part II.

Part II.

A preliminary experiment showed that, after being heated for 6 hours under a vapour pressure of approximately 760 mm., a 60% sucrose solution had undergone inversion to the extent of 20%. Therefore, in order to reduce the time of exposure at high temperatures, the vapour pressure of each solution is determined at only two temperatures, one slightly above and the other slightly below that required to give a vapour pressure of 760 mm. The b. p. under 760 mm. is then determined by interpolation in the logarithmic curve connecting the experimental points (Fig. 2). By this means, the total time of heating is reduced to about 3 hours, and the percentage, in the different solutions, of sucrose inverted, as determined by the polarimeter, is found to lie between 3·71 and 0·82%. The direct determination of invert sugar by Lane and Eynon's method gives the corresponding percentages as 3·61 and 0·87%, respectively, showing that no significant molecular breakdown other than inversion has occurred.

Table V records, for each of the solutions, the concentration, the actual vapour pressure data, and the interpolated temperature, $t_{int.}$, at which $p_s = 760$ mm. Cols. 5 and 6 give the resulting elevation of the b. p. at 760 mm., $E_{exp.}$, and the elevation, $E_{calc.}$, as calculated from

equation (2) and the vapour pressure tables for water given in Landolt–Börnstein's " Tabellen " (1936, Vol. IIIc, p. 2415). The complete inversion data are collected in cols. 7 and 8.

TABLE V.

Elevation of boiling point of pure sucrose solutions at 760 mm. pressure.

p_0 = vapour pressure in mm. at $t_0°$.
$t_{int.}$ = interpolated temperature at 760 mm.
$E_{exp.}$ = elevation of b. p. at 760 mm.
$E_{calc.}$ = elevation of b. p. at 760 mm. calculated from eqn. (2).

Concn., %	p_0	t_0, °C	$t_{int.}$	$E_{exp.}$, 760 mm.	$E_{calc.}$, 760 mm.	I_1*	I_2*
43·23	747·22	100·77	101·25	1·25	$1·30_3$	1·29	1·62
	763·31	101·38					
60·12	758·29	102·77	102·83	2·83	$2·88_8$	1·60	—
	770·59	103·22					
69·27	756·39	104·69	104·79	4·79	$4·66_4$	3·71	3·61
	767·97	105·06					
75·08	751·75	106·45	106·75	6·75	$6·60_7$	2·23	2·25
	763·90	106·89					
79·08	755·19	108·68	108·84	8·84	$8·66_2$	2·78	2·29
	763·05	108·94					
82·74	750·17	111·06	111·45	11·45	$11·44_9$	1·04	1·03
	772·45	111·95					
84·72	748·07	112·96	113·41	13·41	$13·52_9$	1·36	1·43
	766·22	113·65					
86·36	749·28	114·94	115·33	15·33	$15·70_0$	1·56	1·35
	763·92	115·46					
87·87	756·28	117·51	117·62	17·62	$18·18_4$	0·82	0·87
	778·13	118·24					

* I_1 = g. of invert sugar produced per 100 g. of sucrose (by polarimeter).
I_2 = " " " (by Lane and Eynon's method).

The generally accepted values of E at 760 mm. pressure are those of Claassen (*Z. Ver. Deutsch. Zuckerind.*, Techn. T., 1904, **54**, 1159) and of Spengler, Böttger, and Werner (*loc. cit.*, p. 601). In 1941, Tressler, Zimmerman, and Willits (*J. Physical Chem.*, **45**, 1242) carried out a series of boiling-point determinations under atmospheric pressure, designed to give values for the elevation of the boiling point as accurate as are obtainable by a differential modification of the original Beckmann method. The work of all these investigators suffers from the drawback that, although the total weight concentration of the solution purported to be determined at the end of each experiment, no attempt was made to determine the final composition—sucrose and invert sugar—of the solute. Claassen, making determinations of E under atmospheric pressure, reached, in the case of the highest concentrations, temperatures between 120° and 130°, and assuming no inversion, he relied on the polarimeter to give the total concentration. Owing to the opposing effects of the sucrose and invert sugar on the polarimeter reading, the unsuspected presence of even a small percentage of invert sugar would cause a relatively large error in the result. Spengler and Tressler each relied solely upon the refractometer for the same purpose; at lower and medium temperatures, the consequent error might be inconsiderable, since the correction for the presence of invert sugar in the determination of the total solid in a sucrose solution by this method is small. Also, we have demonstrated above that the effect on the vapour pressure of the conversion of sucrose into invert sugar is smaller than would be calculated from the decrease in the mole-fraction of the water in the solution.

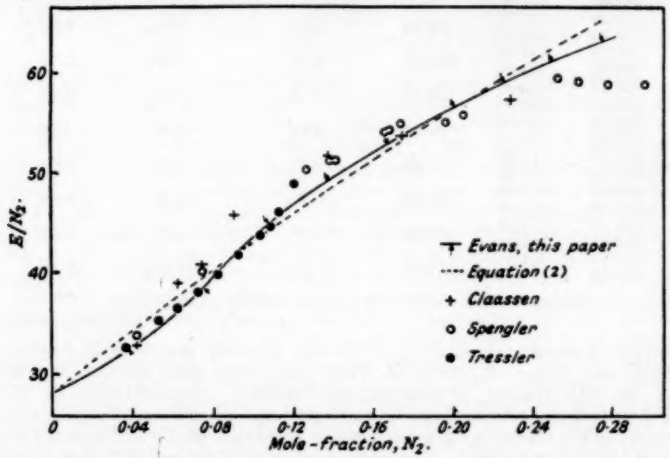
The experiments of Tressler *et al.* were not carried out at 760 mm., but under the prevailing atmospheric pressure. However, the b. p.s of the solution and of the water were determined at the same time and, since the altitude of their laboratory appears to be about 600 feet, the divergence of their values of E from the values at 760 mm. might not be large.

In Spengler's experiments, the pressures were accurately measured and the values of t_0 at 760 mm. were obtained by interpolation, but, in addition to the uncertainty regarding the final percentage of invert sugar, there are well-known errors inherent in the original Beckmann method which Spengler's calibration of his apparatus with pure water (*loc. cit.*, p. 554) would not enable him to estimate. Also, his method of establishing high pressures with solutions of high concentration is open to criticism (*loc. cit.*, p. 552). A great advantage of the method now recorded is that it enables the solutions to be made up very accurately to a known concentration, which not only is maintained throughout the experiment, but also can be checked at the end. Then, as shown above, any one or more of the methods for the estimation of invert can be applied.

To obtain a comparison of the relative consistency of the results of the various methods described, the values of the ratio E/N_2, calculated from the results at 760 mm. of each of the authors concerned, have been plotted against the corresponding mole-fractions of the sucrose (N_2), and the results are illustrated in Fig. 3. The continuous line has been drawn smoothly through all the results (indicated by arrows) from Table V, and the broken line corresponds to the values of E calculated by means of equation (2). The agreement between these lines is quite good, especially when the extent to which the equation has been extrapolated is considered. Not only has it been extrapolated with regard to temperature, but also to some extent

FIG. 3.

Comparison of E/N_2 *against* N_2 *calculated from equation* (2) *and from the results of different investigators.*



with regard to concentration, the equation having been derived from results obtained with solutions ranging in concentration from about 45 to 85% (mole-fraction 0·04—0·20) and over this range it is seen that the values of E/N_2 obtained from the equation and from direct experiment agree closely. The deviation between the two appears to be due to a sigmoid tendency in the experimental curve and, naturally, the simple algebraic relation assumed for the equation cannot reflect this. The results of Tressler *et al.* are in good agreement with the present values except at their highest concentration—about 72% of sucrose—at which they had obviously reached the limit of concentration for which their apparatus was suited. The values given by Claassen and Spengler produce curves of a more pronounced sigmoid tendency. It is difficult to compare the results of these two authors with the present results. The relatively low values of N_2 indicated in the larger proportion of Claassen's results may be referred, as already indicated, to the use of the polarimeter alone for the final determination of concentration. On the other hand, in the case of Spengler's highest concentration, the failure of E to increase consistently with N_2 may be due to the introduction of nitrogen into his apparatus.

Further work on the problems raised by the foregoing results is now in progress in this laboratory.

We wish to thank Messrs. Tate and Lyle for a grant in aid of the work, and Mr. P. Lyle for arranging that this paper shall be submitted for publication. We are also indebted to Professor W. E. Garner, C.B.E., F.R.S., for suggesting the method, and to Professor M. W. Travers, F.R.S., for his valuable assistance in designing the apparatus.

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525. *The Dehydration of Gypsum. Part I. A Study of the Thermal Decomposition of Gypsum by the Adsorption of Oxygen and of Nitrogen at −183°.*

By S. J. Gregg and E. G. J. Willing.

The surface area S ($m.^2$ $g.^{-1}$) of gypsum dehydrated at a succession of temperatures between 100° and 700° has been determined by adsorption of nitrogen and of oxygen. There are maxima for S at *ca.* 180° and *ca.* 280°. In the region *ca.* 150° to *ca.* 350° the area calculated from oxygen adsorption is significantly greater than that calculated from nitrogen adsorption—probably owing to the presence of channels large enough to admit oxygen, but too small to admit nitrogen, molecules. Above 400° the " oxygen " and the " nitrogen " areas agree closely.

More than a thousand papers have been published on calcium sulphate and its hydrates during the past 50 years: very varied results have been obtained and there is much disagreement as to their interpretation. Since the products of dehydration of the dihydrate are " active " solids, possessing a considerable surface area, it was decided to apply to this problem some of the methods used in investigating such solids. The methods selected in the present study include the measurement of the following: adsorption of vapours (carbon tetrachloride at 25°, nitrogen and oxygen at −183°, water vapour at 25°); heat of immersion in an inert liquid (carbon tetrachloride); specific gravity in various liquids; bulk density; and heat of hydration.

The general line of approach has been to prepare a " calcination series " from gypsum; a series of samples of gypsum was taken and each sample was heated to a particular temperature (different for each sample) for a definite length of time; and the properties listed in the previous paragraph were then measured on each sample. The range of temperature covered was from 100° to 700°, at close intervals—10° or so—at the lower end, and wider ones—100° or so—at the higher end of the range.

In the present paper the adsorption of nitrogen and of oxygen on the samples is reported.

Experimental.

Materials.—The gypsum was part of a large lump of transparent gypsum (often called selenite) purchased from Messrs. George and Becker. It was analysed, with the following results (theoretical values for $CaSO_4.2H_2O$ given in parentheses): CaO, 32·56 (32·58); MgO, 0·1; Fe_2O_3, Al_2O_3, SiO_2, absent; SO_3, 45·90 (46·50); CO_2, 0·44; moisture, 0·01; total water, 20·60% (20·93%). The lump was broken up, then ground in a mortar, and finally sieved through I.M.M. sieves so as to obtain a number of definite fractions. In some experiments 40—80-mesh, and in others 80—100-mesh, fractions were used.

The nitrogen and oxygen were obtained from commercial cylinders and were dried by passing through drying tubes containing activated alumina and phosphoric oxide. Before being filled, the storage vessels and drying tubes were thoroughly evacuated and then flushed out several times with dry gas. The oxygen is reported to be 99·4—99·7% pure, the chief impurity being argon, and the nitrogen to be *ca.* 99·9% pure, the chief impurity being oxygen (British Oxygen Co., personal communication).

Procedure.—The volumetric apparatus used for the determination of the adsorption isotherms of oxygen and nitrogen is identical with that already described by Gregg and Sing (*J. Phys. Colloid Chem.*, 1951, **55**, 592) with the addition of an oxygen vapour-pressure thermometer. The whole apparatus was enclosed in a constant-temperature chamber kept at 25—25·5°, to within 0·1°. The saturated vapour pressure was read every time an equilibrium point on the isotherm was determined, and it was found to vary by ±1·5 cm. from time to time, corresponding to a temperature variation of only ±0·2°. The vapour-pressure thermometer showed that the temperature of the bath was, in general, 0·2—0·3° higher than the b.p., owing to superheating.

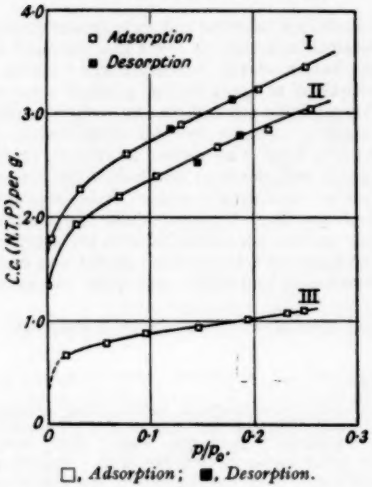
A sample of dehydrated gypsum, 5—6 g., was first prepared by heating the selenite on a thermal balance: this is essentially a crucible hung from the arm of an analytical balance into a furnace maintained at a fixed temperature (for list of references, see Gregg and Winsor, *Analyst*, 1945, **70**, 336). The change in weight on progressive dehydration could thus be followed easily. For samples prepared at temperatures below 300° the heating was continued till the dehydration (to $CaSO_4$) was just complete. At temperatures above this, one hour was used as a standard time; above 300° the time required for decomposition was less than one hour, and became increasingly short as the temperature rose; it would therefore have been difficult at the higher temperatures to arrest the heating when decomposition was

just complete, and it was accordingly deemed more satisfactory to standardise the period of one hour. (Tests, described later, were done to correlate conditions below and above 300°: samples were prepared at 320° and at 404° by heating for different lengths of time between 30 minutes and 24 hours.)

As soon as possible after preparation, the sample was weighed, with the minimum exposure to air, into the adsorbent bulb. The bulb was then attached to the volumetric apparatus by its ground-glass joint, a heating unit maintained at 85—90° was placed around it, and the sample was out-gassed (by means of a mercury condensation pump, backed by a "Speedivac") for 2—2½ hours. The heater was then removed, and replaced by a liquid-oxygen bath, the level of the oxygen being kept at a fixed mark.

FIG. 1.

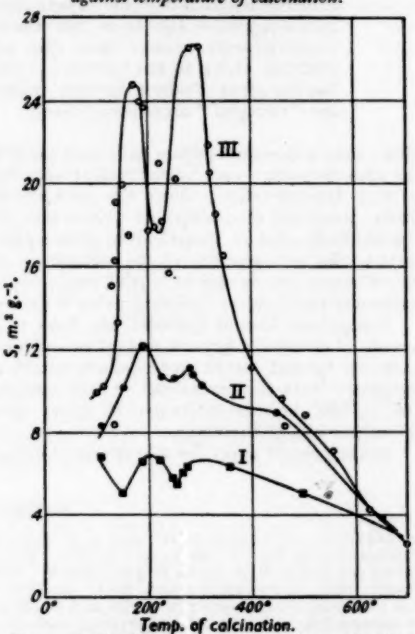
Typical adsorption isotherms of nitrogen at — 183° on gypsum dehydrated at different temperatures T. *Curve I*, T = 190°; *Curve II*, T = 287°; *Curve III*, T = 655°.



□, *Adsorption*; ■, *Desorption*.

FIG. 2.

Graph of surface area S *of dehydrated gypsum against temperature of calcination.*



(S *was calculated from adsorption isotherms of carbon tetrachloride for Curve I, of nitrogen for Curve II, and of oxygen for Curve III.*)

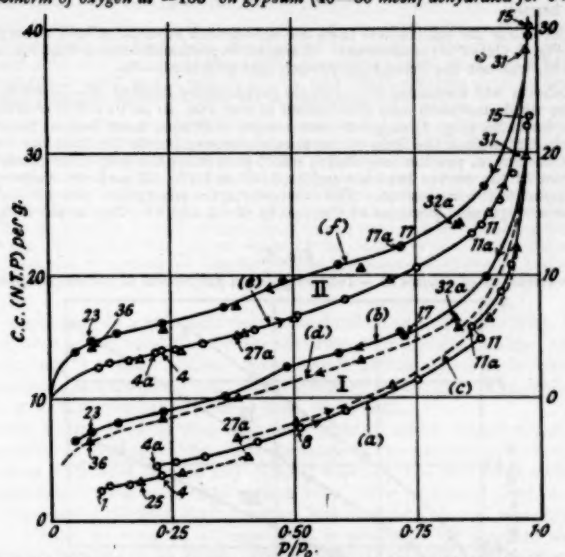
Results.—Nitrogen isotherms. Because of the high value of the saturated vapour pressure of nitrogen at 90·5° K., the highest relative pressure that could be attained with this gas was *ca.* 0·25. Consequently, no complete isotherms were obtainable. The time required for equilibration was less than 15 minutes; and the isotherms were *reversible*, *i.e.*, points obtained by withdrawing gas fell on the same curve as those obtained when gas was being added.

Typical isotherms are given in Fig. 1. Because of the restricted range, it is not possible to decide, from a mere inspection of the isotherms themselves, to what type they belong (cf. Brunauer, Deming, Deming, and Teller, *J. Amer. Chem. Soc.*, 1940, **62**, 1723). However, comparison with the two oxygen isotherms, which were carried up to higher relative pressures, leaves little doubt that they are of Type II.

Oxygen isotherms. With two of the samples the measurements were carried up to high relative pressures. The isotherms appeared to be quite definitely of Type II, but presented certain unusual features. The first experiment was carried out on a sample of 40—80 mesh heated for 6 hours at 144°, and the isotherm is shown in Fig. 3 (Curve II). Points 1—4 were determined on the first day; the time required for equilibration was 1—1½ hours. Overnight some more adsorbate was taken up with a slight reduction in pressure (point 4*a*). On continuation of the admission, points 4*a*—11 fell on a smooth curve. When the system was again left overnight another drift was observed (point 11*a*). The curve was continued on the third day to a relative pressure of 0·98, whereupon withdrawal of gas was begun. As will be seen, a hysteresis loop was obtained, and it did not close even at the lowest pressure reached. It is particularly noteworthy that the small overnight drift (between points 17 and

FIG. 3.

Adsorption isotherm of oxygen at −183° on gypsum (40—80 mesh) dehydrated for 6 hours at 144°.

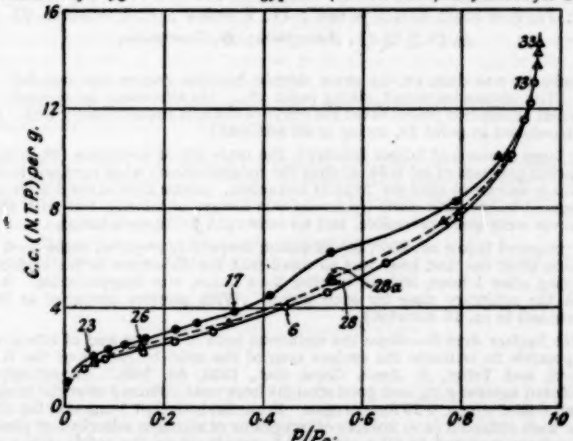


p = *equil. pressure of oxygen,* p_0 = *satd. vapour pressure at* −183°. (*The ordinates of isotherm II have all been increased by* 10 *c.c. g.*$^{-1}$.) *The experimental points were numbered in the order in which they were determined. The numbers of the points commencing from the origin in each case are : Curve Ia* : 1, 2, 3, 4, 4*a*, 5, 6, 7, 8, 9, 10, 11, 11*a*, 12, 13, 14, 15; *Curve Ib* : 24, 23, 22, 21, 20, 19, 18, 17*a*, 17, 16; *Curve Ic* : 25, 26, 27, 27*a*, 28, 29, 30, 31; *Curve Id* : 36, 35, 34, 33, 32*a*, 32, 31; *Curve IIe* : 1, 2, 3, 25, 4*a*, 4, 5, 26, 6, 27*a*, 27, 7, 28, 8, 9, 29, 10, 11*a*, 11, 3*a*, 12, 13, 31, 14, 15; *Curve IIf* : 24, 23, 36, 32, 21, 35, 20, 34, 19, 18, 33, 17*a*, 17, 16.

○ △, *Adsorption*; ● ▲, *Desorption.*

FIG. 4.

Adsorption isotherm of oxygen at − 183° *on gypsum (20—30 mesh) dehydrated for 1 hour at 465°.*



The experimental points were numbered in the order in which they were determined. The numbers of the points, commencing from the origin in each case are : ○, 1—13; ●, 24—14; △, 25—33; ▲, 34.

○ △, *Adsorption*; ● ▲, *Desorption.*

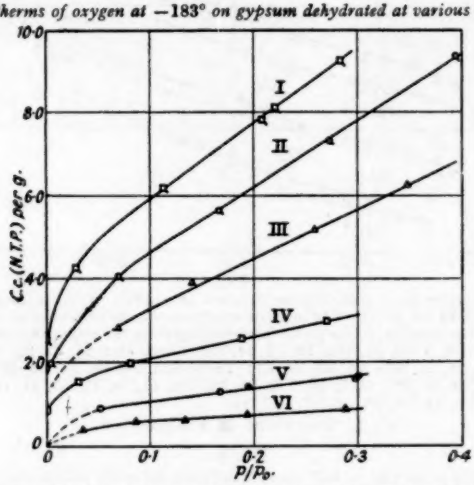
17*a*) led to an *increase* in the amount adsorbed; this is opposite to the behaviour normally encountered in adsorption work, where the amount adsorbed *decreases* if the system is left overnight at any point on the desorption branch.

If the overnight drifts are subtracted from all subsequent adsorption and desorption points, the smooth isotherm (Fig. 3, Curve II) is obtained. A second experiment showed that the *adsorption* branch could be reproduced, but not the desorption branch (see points 25—36).

The second isotherm was measured on a sample prepared by heating 20—30-mesh gypsum at 465° for one hour. The whole isotherm was determined in one run, so as to avoid overnight drifts. The appearance of the isotherm (Fig. 4) suggests that a slow drift was, nevertheless, present the whole of the time; and that, although in the interval between successive points the drift was too small to affect the smoothness of the curve, yet the cumulative effect prevented the loop from closing near point 16. The near-parallelism of the curves between points 2—6 and 17—23, and the shape of the hysteresis loop, lend some support to the suggestion. The difference in the adsorption values at any given pressure, between the two nearly parallel portions of the curves (2—6 and 17—23), would then give a measure of the drift.

FIG. 5.

Adsorption isotherms of oxygen at −183° *on gypsum dehydrated at various temperatures* T.



I, $T = 299°$; *II*, $T = 254°$; *III*, $T = 345°$; *IV*, $T = 505°$; *V*, $T = 630°$; *VI*, $T = 700°$.

△, ○ □ ◯ ◻, *Adsorption*; ●, *Desorption*.

A second experiment was done on the same sample, but the system was now left overnight after point 28 (Fig. 4). The uptake increased, giving point 28*a*. On allowance being made for this drift as before, the subsequent adsorption points fitted the curve of the first run extremely well. (The desorption curve had to be abandoned at point 34, owing to an accident.)

In view of the large amount of labour involved, the main set of isotherms (36 in number) was not taken beyond a partial pressure of *ca.* 0·35, so that the measurements were confined to the range where the B.E.T. equation is known to hold for Type II isotherms. Some typical results are shown in Fig. 5. With samples prepared below 500° some hysteresis was always obtained; but with samples prepared above 500° the curves were quite reversible, and no overnight drifts were noted.

With samples prepared below *ca.* 250°, equilibration frequently required some 2—3 hours; but the change in adsorption after the first hour was so small that the difference in the surface area obtained by taking the reading after 1 hour, instead of after 2—3 hours, was inappreciable. Accordingly, one hour was fixed as the arbitrary time for each point. (With samples prepared at 500° and above, equilibrium was reached in *ca.* 15 minutes.)

Estimation of the Surface Area S.—Since the isotherms both of oxygen and of nitrogen may be taken as Type II, it is possible to estimate the surface area of the solid by means of the B.E.T. procedure (Brunauer, Emmett, and Teller, *J. Amer. Chem. Soc.*, 1938, **60**, 309). Accordingly, the quantity $p/v(p_0 - p)$ was plotted against p/p_0, and good straight lines were obtained over the range $0{\cdot}05 < p/p_0 < 0{\cdot}30$ for oxygen, and $0{\cdot}05 < p/p_0 < 0{\cdot}25$ for nitrogen. From the intercept $1/v_m c$ and the slope $(c-1)/v_m c$, values for v_m and c were obtained (v = volume of oxygen or of nitrogen adsorbed at pressure p, reduced to N.T.P.; v_m = volume required to fill a completed monolayer on the solid; c is a constant of the B.E.T. equation given by $c = e^{(E_1 - \lambda)/RT}$, where E_1 is the heat of adsorption in the first molecular layer, λ is the latent heat of condensation, and R and T have their usual meanings. To calculate S

from v_m, a value had to be assumed for the cross-sectional area A_0 of the molecules of the adsorbate; the figures here taken were $A_0 = 14{\cdot}1$ A.^{2} for oxygen, and $A_0 = 17{\cdot}0$ A.^{2} for nitrogen (cf. Emmett and Brunauer, *J. Amer. Chem. Soc.*, 1937, **59**, 1553).

In Fig. 2 the area S is plotted against the temperature of preparation (Curve II for nitrogen, Curve III for oxygen). As will be seen, the areas from the nitrogen are much smaller than those from the oxygen data over the range 150—350°, whereas from 400° upwards there is close agreement. The oxygen curves show pronounced maxima in the regions of 180° and 280°; maxima occur in the nitrogen curves also at about the same temperatures, but they are much less pronounced.

The effect on S of varying the time of heating was studied at two temperatures by reference to the oxygen isotherms. At 404° the effect is small: samples prepared by heating for ¼ hour, 1 hour, and 24 hours had surface areas of 11·1, 11·2, and 10·2 m.^{2} g.$^{-1}$, respectively. At 320° the effect was more marked, successive experiments with times of 1 hour, 1 hour, ¼ hour, ¼ hour, and 24 hours giving areas of 20·5, 21·8, 13·7, 17·2, and 13·8 m.^{2} g.$^{-1}$, respectively. Reference to Curve III of Fig. 2 will show that 320° is on a steeply falling branch of the curve of temperature against area, where one would expect an unusually marked dependence of area on the conditions of preparation, including the time of heating. One is justified in concluding that the general shape of the curves of Fig. 2 would not be significantly different had one been able to prepare all the samples, at temperatures above as well as below 300°, by heating " just to decomposition."

Discussion.—According to a principle due to Hedvall (*Chem. Review*, 1934, **15**, 139), a solid is brought into a more active condition by the occurrence of a phase change, which loosens the lattice (" Auflockerung "). An " active " solid is one which dissolves in solvents, or reacts more rapidly than the corresponding solid in its ordinary condition. The activity may be due to lattice strain, or to the presence of a large area, or to both.

In systems such as the present one, the phase change which brings about activation is a thermal decomposition. Acting in the opposite sense is the process of *sintering*; understood in its broadest sense, this term implies any process whereby the sample of solid tends towards a state with no strain and with a minimal surface area. The process is most probably complex, and comprises a number of distinct mechanisms; but in general it will be accelerated by a rise in temperature. Hence, as soon as a larger area is produced by thermal decomposition, it will begin to diminish because of sintering. Since the two processes—activation and sintering—will in general have different characteristics and different temperature coefficients, the occurrence of a maximum in S at a particular temperature is understood.

Evidence is presented in later parts of this series that the phase change responsible for the first maximum at 180° is Gypsum ⟶ Hemihydrate + Water, and for the second Hemihydrate ⟶ Anhydrite + Water.

The difference in the values of S obtained by oxygen and by nitrogen adsorption respectively is believed to originate in the sizes of the channels which are formed during the dehydration; since the oxygen molecule is smaller ($A_0 = 14{\cdot}1$ A.^{2}) it can penetrate into pores from which the nitrogen molecule ($A_0 = 17{\cdot}0$ A.^{2}) is excluded. This view is consistent with (*a*) the longer time for equilibration with oxygen, and (*b*) the peculiar hysteresis in which, it will be recalled, the amount adsorbed *increased* if the system was left overnight even on the *desorption* branch. If this explanation is correct, it will be inferred from Curves II and III of Fig. 2 that the area of the walls of these finer pores is very considerable, being of the same order as the area accessible to the nitrogen molecules only. The results with carbon tetrachloride as adsorbate, reported in Part II, are in conformity with this explanation.

Our thanks are due to the I.C.I. Research Fund for providing certain apparatus; and one of us (E. G. J. W.) acknowledges receipt of a Further Education and Training Grant from the Ministry of Education, during the tenure of which this work was carried out at University College, Exeter.

University College, Exeter.
National Coal Board Central Research Establishment,
Stoke Orchard, Nr. Cheltenham, Glos.

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526. *The Dehydration of Gypsum. Part II.* A Study of the Thermal Dehydration of Gypsum by Means of the Adsorption of Carbon Tetrachloride Vapour at 25°.*

By S. J. Gregg and E. G. J. Willing.

The adsorption of carbon tetrachloride vapour has been measured at 25° on samples of gypsum dehydrated at a succession of temperatures between 100° and 700°. The adsorption isotherms are of B.E.T. Type II with a small value of the parameter c, so the surface area S cannot be determined by application of the B.E.T. procedure. Since, however, all the isotherms can be superposed by adjustment of the scale of the adsorption ordinates, the *relative* values of S can be obtained from adsorption at a given pressure. The curve of S against temperature of preparation resembles those obtained in Part I, from oxygen and nitrogen adsorption, with its maxima now at *ca.* 200° and 300—350°.

In Part I,* the products of the thermal decomposition of gypsum were studied by means of the adsorption of nitrogen and of oxygen. A similar study, by using the adsorption of carbon tetrachloride vapour, is now described.

Experimental.

Materials.—The gypsum was taken from the same lump as that described in Part I. The individual samples were prepared in an exactly similar manner, the range of temperatures being again *ca.* 100—700°, spaced at close intervals at the low-temperature end of the range and at wider ones at the high-temperature end.

The carbon tetrachloride was purified by Fieser's method (" Experiments in Organic Chemistry," 1941, p. 365).

Apparatus.—For the determination of the adsorption, an improved design of the automatically recording sorption balance (Gregg and Wintle, *J. Sci. Instr.*, 1946, **23**, 259) was used. The sample of solid is contained in a bucket, hung in an enclosed space which can be evacuated or can be filled with a known vapour; the change in weight of the solid as it takes up or loses vapour is registered automatically as a function of time, on a revolving drum. The balance case was joined on to a system for the storage of the carbon tetrachloride and its controlled admission to the sorption balance; this system had no taps and the movement of vapour was controlled by suitably raising and lowering mercury levels (cf. Gregg and Sing, *J. Phys. Colloid Chem.*, 1951, **55**, 597). The lubrication of the ground-glass joints on the sorption balance proper was by Dow–Corning High Vacuum Silicone Grease; as an additional precaution the joints were also coated on the outside first with black wax (Edwards's Wax W) and on top of that Everett's No. 1 Hard (m. p. 57°) Vacuum Wax.

Procedure.—The dehydrated gypsum (3—4 g.) was weighed into the bucket of the sorption balance, and an asbestos plug inserted above it to prevent spurting. The outer limb of the balance case was placed in position, and an electric oven maintained at 80—90° put round it. Pumping commenced, and was continued until no further change in weight was recorded on the drum (*ca.* 2 hours). The connection to the pump was then closed by a mercury cut-off and the oven was replaced by a water-bath kept at 25° ± 0·05°. After one hour (for attainment of thermal equilibrium) a charge of carbon tetrachloride was admitted, and the increase in weight registered itself on the recording drum. When the weight had become constant, the pressure p in the dead space was read on a mercury manometer by means of a cathetometer. A series of admissions was made until the pressure reached about 90% of the saturated vapour pressure. Controlled withdrawals were then made in a similar manner, by opening the balance system to the pumps through a mercury cut-off.

Results.—The adsorption isotherms obtained are of B.E.T. Type II, but the initial, steeply rising, portions are so short that they approximate to Type III (Brunauer, Emmett, and Teller, *J. Amer. Chem. Soc.*, 1938, **60**, 309). This implies that the value of the B.E.T. parameter c must be close to unity (cf. Gregg and Jacobs, *Trans. Faraday Soc.*, 1948, **44**, 574); its exact value cannot be determined owing to insufficiency of data at low pressures. (Many points are necessary to determine c when $c \simeq 1$.)

A representative example is given in Fig. 1. The hysteresis loop is typical, and was obtained in all cases except one; it always closed at low pressure. Equilibrium was attained very rapidly, in less than 5 minutes at all points on the adsorption and desorption branches: check readings were sometimes taken by leaving the system for 4 or 5 hours, and in no case was any increase in adsorption registered. This would seem to exclude the possibility of slow penetration into narrow channels, such as might perhaps have been expected. It suggests that any holes left in the solid are either smaller than the diameter of a carbon tetrachloride molecule (*ca.* 5·5 A.) or are considerably larger—*i.e.*, that they are not close to 5 A. in diameter.

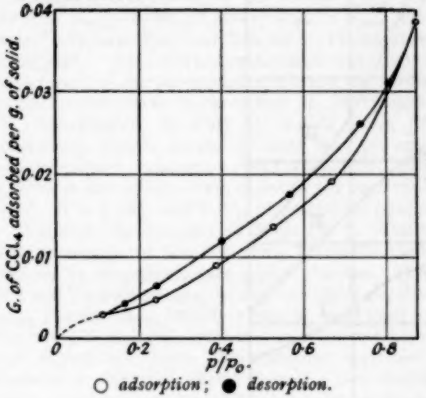
* Part I, preceding paper.

All the carbon tetrachloride could be pumped off at 25° in about an hour, a behaviour characteristic of physical adsorption and showing that chemisorption or penetration into the lattice (*e.g.*, solid solution) is absent. In principle, therefore, the carbon tetrachloride isotherm can be used for the determination of the surface area of the solid.

Calculation of Surface Area S.—It had been hoped that the carbon tetrachloride isotherms would be of B.E.T. Type II with a large value of c (corresponding to a sharp knee at the low-pressure end). It would then have been possible to calculate S with accuracy by the usual B.E.T. procedure; but this procedure is known to fail when $c \triangleq 1$, as in the present case. However, it fortunately turned out that

FIG. 1.

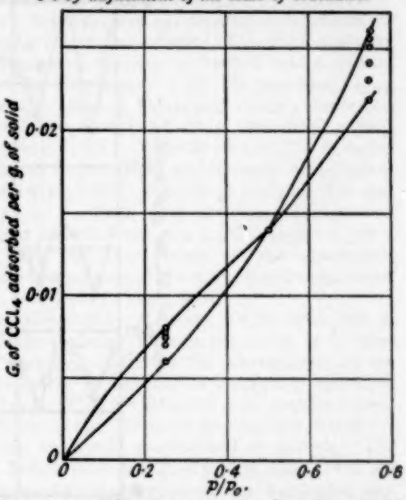
Adsorption isotherm of carbon tetrachloride vapour at 25° on gypsum (80—100 mesh) dehydrated for 2 hours at 256°.



○ *adsorption*; ● *desorption.*

FIG. 2.

Superposition of the adsorption isotherms of carbon tetrachloride vapour at 25° on samples of gypsum dehydrated at various temperatures. The isotherms were brought to coincidence at $p/p_0 = 0{\cdot}5$ *by adjustment of the scale of ordinates.*



At $p/p_0 = 0{\cdot}25$, *the points (reading downwards) refer to the samples dehydrated at the following temperatures:* 110°, 188°, 222°, *and* 288°; 358°, 297°; 152°, 245°; 498°, 700°; 256°; *and at* $p/p_0 = 0{\cdot}75$, *at the following temperatures:* 152° *and* 245°; 288° *and* 358°; 110°, 188°, 250°, *and* 256°; 700°; 222°; 498°.

all the isotherms obtained can be superposed within reasonably close limits, by a mere adjustment of the adsorption ordinates. This implies that the curves are all of the *same shape*. Hence, independently of any particular theory of the isotherm, it is possible to obtain *relative* values of S by taking one of the isotherms as standard, and finding the factor f by which to multiply the ordinates of the others to bring them to coincidence. The point is illustrated by reference to Fig. 2. The factor has there been found by taking the 245° isotherm as standard, and putting $f = 1/x_{0\cdot5}$, where $x_{0\cdot5}$ is the uptake on any other isotherm when $P = 0{\cdot}5$; the value of the uptake at $P = 0{\cdot}25$ and at $P = 0{\cdot}75$ has then been multiplied by f to get derived adsorptions for each of the other temperatures ($P = p/p_0$, where p_0 = saturated vapour pressure). As will be seen, the points at these last two relative pressures do not diverge by more than $\pm 10\%$ or so compared with the original divergencies of some three-fold.

Alternatively expressed, one may take the value of the carbon tetrachloride adsorption, say x_p, of a sample at a given value of P, as proportional to S. In Fig. 3, x_p has been plotted against the temperature of preparation for $P = 0{\cdot}25$, $P = 0{\cdot}5$, and $P = 0{\cdot}75$. The three curves so obtained are of the same shape and they all show maxima at *ca.* 200° and at 300—350°. (The points were not sufficiently close together to give better precision than this).

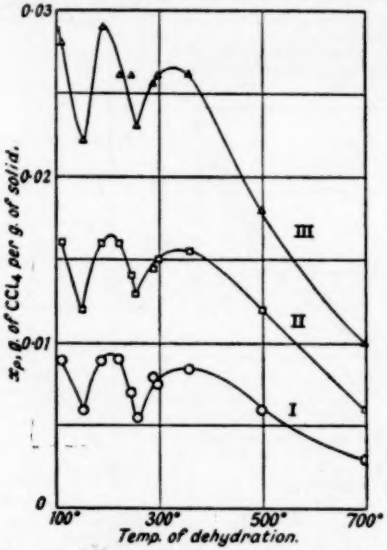
To obtain absolute values of S from the carbon tetrachloride isotherms it is only necessary to know the absolute area for one sample, and the others can then be calculated thence by comparison of the uptakes at a given pressure, as just explained. The 700° was taken as the reference sample, and the area given by the nitrogen and the oxygen isotherms is assumed correct; this sample has been selected as being the most sintered and accordingly the most free from fine pores—which might, for example, exclude

the carbon tetrachloride molecule. The curve of (absolute) S against temperature of preparation, thus obtained, is given in Fig. 2 of Part I, along with that calculated from the oxygen and the nitrogen isotherms.

Discussion.—From Fig. 2 of Part I, it will be seen that the maxima in the curve derived from carbon tetrachloride do not quite coincide with those from the oxygen- and the nitrogen-adsorption data; the difference may well originate in the difference in molecular size between carbon tetrachloride and the other two gases: since the carbon tetrachloride molecule is larger than the others it would be excluded from pores which are open to them. This suggestion is consistent with the lower value of S obtained from carbon tetrachloride adsorption.

FIG. 3.

Plot of x_p against the temperature of dehydration of the gypsum.



(x_p = *amount of* CCl_4 *adsorbed by the sample, at relative pressure* P.) *Curve I*, P = 0·25; *Curve II*, P = 0·50; *Curve III*, P = 0·75.

The origin of the maxima is believed to be the same as that of the nitrogen and oxygen curves: the lower-temperature maximum is due to the reaction Gypsum ⟶ Hemihydrate + Water, and the higher-temperature one to Hemihydrate ⟶ Anhydrite + Water. The detailed evidence for this view will be presented in Part III.

Our thanks are due to Messrs. Standard Telephones and Cables for undertaking the construction of the automatic recorder; and particularly to Mr. M. F. Wintle (late of that Company) and to the Company's workshop staff for their care in the design and the execution of the new features of the instrument. We also thank the Imperial Chemical Industries Limited for the provision of apparatus through their Research Fund. The work was carried out at University College, Exeter.

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527. *The Application of the Method of Molecular Rotation Differences to Steroids. Part XVII.* The Action of Mercuric Acetate on* iso-*Dehydrocholesteryl* p-*Nitrobenzoate.*

By D. H. R. Barton and W. J. Rosenfelder.

When treated with mercuric acetate in chloroform–acetic acid and then hydrolysed by alkali, *iso*dehydrocholesteryl *p*-nitrobenzoate furnishes a mixture of cholesta-7 : 9(11)-diene-3β : 6α- and -3β : 6β-diols.

In an extensive study of the action of mercuric acetate and lead tetra-acetate on *iso*dehydrocholesteryl *p*-nitrobenzoate (I; R = *p*-NO_2·C_6H_4·CO) Windaus and his collaborators (*Annalen*, 1942, **552**, 135, 142) prepared a number of interesting labile derivatives. The main material produced by reaction with mercuric acetate and subsequent alkaline hydrolysis was a doubly unsaturated diol, $C_{27}H_{44}O_2$, m. p. 228°, $[\alpha]_D$ −49° (in chloroform), −51° (in pyridine), $\lambda_{max.}$ 248 mμ., $\varepsilon_{max.}$ 15,000 (in ether) [Calc. : $\lambda_{max.}$ 255 mμ. in ethanol, Fieser and Fieser's correction table ("Natural Products Related to Phenanthrene," Reinhold Publ. Corp., 3rd Edn., p. 184) being used]. This was assigned the constitution cholesta-7 : 9(11)-diene-3β : 6-diol (II; R = H) on the basis of the absorption spectrum, the structure of its precursor, and it ready dehydration by acetic anhydride to cholesta-5 : 7 : 9(11)- and -6 : 9(11) : 8(14)-trien-3β-yl acetates (III and IV respectively). In Part II of this series (Barton, *J.*, 1946, 512) this constitution was questioned, mainly on the grounds that the rotation recorded was too highly negative for a 6-hydroxylated cholesta-7 : 9(11)-dien-3β-ol and that the wave-length of the absorption maximum should have been closer to 242 mμ., the value for ergosterol D and 22-dihydroergosterol D (V; R = C_9H_{17} and C_9H_{19} respectively) (Barton and Cox, *J.*, 1949, 219).

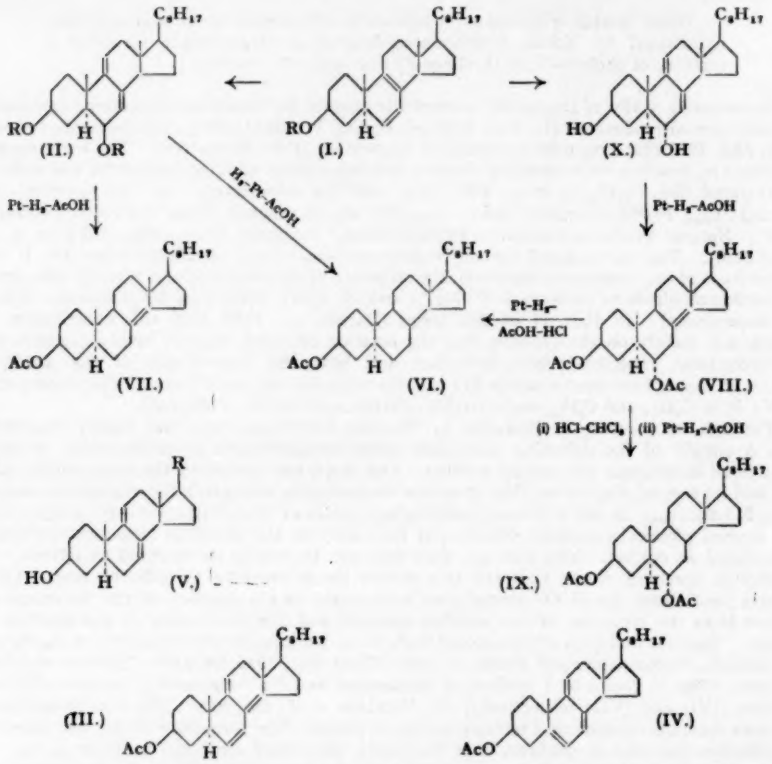
Through the courtesy of Professor A. Windaus (Göttingen) who very kindly provided us with a supply of the difficultly accessible *iso*dehydrocholesteryl *p*-nitrobenzoate, it became possible to investigate this subject further. Our work has confirmed the homogeneity of the diol and its ease of diacylation, but there are discrepancies as regards the absorption spectrum ($\lambda_{max.}$ 245 mμ., $\varepsilon_{max.}$ 18,300 in *ethanol*) and rotation ($[\alpha]_D$ −41° in pyridine but +20° in chloroform). The revised physical constants remove our objections to the Windaus formulation which we now regard as correct. The relevant argument can be briefly summarised as follows. The absorption spectrum shows that the two double bonds must be in different rings. Of the various possibilities the $\Delta^{7:9(11)}$-formulation is adequate for the position of the maximum and follows from the structure of the starting material and the mechanism of the reaction (see below). That the diol is an allylic alcohol is shown by the smooth hydrogenolysis of its diacetate (cf. *inter al.*, Wintersteiner and Ruigh, *J. Amer. Chem. Soc.*, 1942, **64**, 2456; Henbest and Jones, *J.*, 1948, 1798) to give a 3 : 1 mixture of cholestanyl and "α"-cholestenyl [cholest-8(14)-enyl] acetates [(VI) and (VII) respectively] (cf. Windaus *et al.*, *loc. cit.*). The ease of diacylation excludes formulæ containing a tertiary hydroxyl group. The formation of the two trienes on dehydration (see above) confirms that the easily eliminated hydroxyl must be at $C_{(6)}$. As will be clear from the sequel the diol must have the β-configuration at $C_{(6)}$ and therefore is represented by (II; R = H).

In our hands benzoylation of the mother-liquors from the preparation of the cholesta-7 : 9(11)-diene-3β : 6β-diol gave the dibenzoate, m. p. 184—185°, $[\alpha]_D$ +144° (in chloroform), of a further isomeric diol. The latter was obtained from the dibenzoate by alkaline hydrolysis in the usual way and was further characterised as the diacetate, m. p. 126—127°, $[\alpha]_D$ +126° (in chloroform), $\lambda_{max.}$ 242 mμ., $\varepsilon_{max.}$ 19,500 (in ethanol). On catalytic hydrogenation in acetic acid in presence of platinum the diacetate gave cholest-8(14)-ene-3β : 6α-diol diacetate (VIII), m. p. 141—142°, $[\alpha]_D$ +28° (in chloroform). The constitution assigned to the latter is based on (*a*) its rotation and ultra-violet absorption spectrum (see Experimental), (*b*) its catalytic hydrogenation in acetic acid containing dissolved hydrogen chloride to cholestanyl acetate (VI), and (*c*) its conversion into cholestan-3β : 6α-diol diacetate (IX) on rearrangement of the double bond by hydrogen chloride in chloroform, followed by further hydrogenation. This formulation and the interpretation of the reactions are also based on sound analogy (*e.g.*, Fieser and Fieser, *op. cit.*, p. 240 *et seq.*). Isolation of this compound, coupled with the absorption

* Part XVI, *J. Amer. Chem. Soc.*, 1950, **72**, 1633.

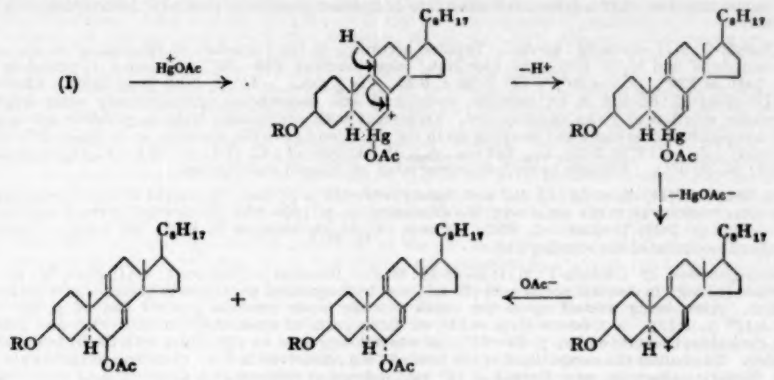
spectrum of the parent dienediol diacetate and the known structure of its precursor, shows that the dienediol must be cholesta-7 : 9(11)-diene-3β : 6α-diol (X).

Cholesta-7 : 9(11)-diene-3β : 6α-diol is very probably identical with an incompletely characterised diol obtained by Windaus *et al.* (*loc. cit.*) by the action of lead tetra-acetate on *iso*dehydrocholesteryl *p*-nitrobenzoate. In agreement with this view, Windaus *et al.* reported that on treatment with acetic anhydride their diol gave a mixture of (III) and (IV).



The mechanism of the action of mercuric acetate on *iso*dehydrocholesteryl *p*-nitrobenzoate appears worthy of brief mention. It would seem that this is best regarded as electrophilic attack of $HgOAc^{+}$ on the diene system, followed by loss of a proton. The organomercuri-complex thus formed could conceivably be the actual product of the reaction, the two cholesta-7 : 9(11)-dienediols resulting therefrom only by hydrolysis on working up. Against this theory is the fact that lithium aluminium hydride, which cleaves, for example, the Hg—C bond of diphenylmercury very smoothly, gives the same reaction products as the ordinary alkaline hydrolysis with no indication of the formation of cholesta-7 : 9(11)-dien-3β-ol (V; R = C_8H_{17}). The first stage of the reaction that can be studied (see Experimental) leads to the introduction of a non-acylatable group at $C_{(6)}$ which is, presumably, the acetoxy-group. The reaction sequence can be formulated as indicated below (R = *p*-NO_2·C_6H_4·CO throughout).

Windaus *et al.* (*loc. cit.*) reported the formation of a substance, $C_{34}H_{43}O_5N$, as a by-product of the action of mercuric acetate on *iso*dehydrocholesteryl *p*-nitrobenzoate. This compound was isolated before the usual alkaline hydrolysis. We have confirmed these findings and have shown that the newly introduced oxygen function cannot be acetylated readily or oxidised by



$$HgOAc^- + \overset{+}{H}gOAc \longrightarrow Hg_2(OAc)_2$$

chromic acid under very mild conditions. The infra-red absorption spectrum showed the hydroxyl band at 2·87 μ. and the compound is clearly a tertiary alcohol. Its constitution and mode of formation remain obscure.

Experimental.

M. p.s are uncorrected. Unless specified to the contrary rotations were determined in chloroform at room temperature 15—25°. For rotation measurements all specimens were dried *in vacuo* at 20° below their m. p.s or at 110°, whichever was the lower. Values of $[\alpha]_D$ have been approximated to the nearest degree.

Savory and Moore's alumina for chromatography was used in all cases. Light petroleum refers to the fraction of b. p. 40—60°.

In the text below the phrase "in the usual way" refers to dilution with water, extraction with ether, washing successively with aqueous potassium hydroxide solution, aqueous hydrochloric acid, and water, followed by evaporation of the ethereal solution *in vacuo*. Where necessary water was removed from the residue by azeotropic distillation *in vacuo* with benzene as entrainer.

Alkaline hydrolyses were effected by using several equivalents of potassium hydroxide and refluxing the reactants for 30—60 minutes in methanolic or dioxan–methanolic solution depending on the solubility requirements of the ester.

Absorption spectra were measured in ethanol by a Unicam S.P. 500 spectrophotometer.

Action of Mercuric Acetate on iso*Dehydrocholesteryl* p-*Nitrobenzoate.*—*iso*Dehydrocholesteryl *p*-nitrobenzoate was treated with mercuric acetate in chloroform–acetic acid according to the directions of Windaus, Riemann, and Zühlsdorff (*Annalen*, 1942, **552**, 135) except that it was found advantageous to leave the reactants for only 1 hour before working them up. As reported by Windaus, Riemann, and Zühlsdorff (*loc. cit.*) the crude reaction product, on treatment overnight with acetone, gave long pale yellow needles of a compound, $C_{34}H_{43}O_5N$, which, recrystallised from ethyl acetate, had m. p. 209—210°, $[\alpha]_D$ −121° (*c*, 2·11), $\lambda_{max.}$ 258 and 312 mμ., $\varepsilon_{max.}$ 16,000 and 13,500 respectively (Found: C, 74·8; H, 7·7. Calc. for $C_{34}H_{43}O_5N$: C, 74·8; H, 7·9%). After attempted acetylation (acetic anhydride–pyridine at room temperature overnight) or benzoylation (similar conditions but with benzoyl chloride) the compound was recovered unchanged. After treatment of the compound (67·5 mg.) in AnalaR benzene (2 ml.) with a solution (2·04 ml.) of AnalaR chromium trioxide (101·2 mg. in 25 ml.) in AnalaR acetic acid at 0° overnight, not all the chromic acid had been consumed. Working up in the usual way gave 34 mg. of unchanged starting material.

After removal of the *p*-nitrobenzoate referred to above unsuccessful attempts were made to crystallise the residual compounds. The vacuum-dried residue had $[\alpha]_D$ −14° (*c*, 1·24), $\lambda_{max.}$ 253 mμ., $\varepsilon_{max.}$ about 20,000. After attempted acetylation (as above) the rotation was unchanged ($[\alpha]_D$ −16° (*c*, 2·18)) and therefore the products at this stage of the reaction cannot have a free hydroxyl group at $C_{(6)}$.

Two methods of working up the mixed *p*-nitrobenzoates were adopted: (*a*) hydrolysis with methanolic potassium hydroxide according to the directions of Windaus, Riemann, and Zühlsdorff (*loc. cit.*); and (*b*) reduction by an excess of lithium aluminium hydride in ethereal solution (1 hour's reflux), followed by working up in the usual way. Both procedures gave the two diols referred to below, but the yield was better on following procedure (*a*).

In a quantitative experiment, *iso*dehydrocholesteryl *p*-nitrobenzoate (1·74 g.), treated with mercuric acetate (4·0 g.), gave mercurous acetate (2·16 g., 114% on the assumption of a 1 : 1 molar relationship).

This again indicates that soluble mercuriacetate complexes cannot be isolatable intermediates in the reaction.

Cholesta-7 : 9(11)-*diene*-3β : 6α-*diol*.—Isolated according to the procedure of Windaus *et al.* (*loc. cit.*), this compound had m. p. 203—204° (decomp.; slow heating), 226—227° (decomp.; if placed in the m. p. bath at 220°), $[\alpha]_D$ +20°, +17° (both *c*, 0·33; *l* = 2 dm.), −41° (*c*, 2·12) in pyridine. Cholesta-7 : 9(11)-diene-3β : 6β-diol is an unstable compound and decomposes spontaneously when kept in chloroform solution at room temperature. Treatment with acetic anhydride in pyridine solution at room temperature overnight and working up in the usual way gave the *diacetate*, m. p. 131—132° (from methanol), $[\alpha]_D$ −115° (*c*, 2·21), $\lambda_{max.}$ 245 mμ., $\varepsilon_{max.}$ 18,300 (Found : C, 77·1; H, 10·1. $C_{31}H_{44}O_4$ requires C, 76·8; H, 10·0%). Alkaline hydrolysis regenerated unchanged starting diol.

Cholesta-7 : 9(11)-diene-3β : 6β-diol and benzoyl chloride in pyridine overnight at room temperature gave, after working up in the usual way, the *dibenzoate*, m. p. 149—150° (from ethyl acetate–methanol), $[\alpha]_D$ −132° (*c*, 2·22) (Found : C, 80·7; H, 8·8. $C_{41}H_{52}O_4$ requires C, 80·8; H, 8·6%). Alkaline hydrolysis regenerated the starting diol.

Hydrogenation of Cholesta-7 : 9(11)-*diene*-3β : 6β-*diol Diacetate*.—Cholesta-7 : 9(11)-diene-3β : 6β-diol diacetate (40 mg.) in AnalaR acetic acid (20 ml.) was hydrogenated overnight in presence of a platinum catalyst. After being worked up in the usual way the crude reaction product had m. p. 95—96°, $[\alpha]_D$ +12° (*c*, 1·12). A mixture ($[\alpha]_D$ +13°) of three parts of cholestanyl acetate with one part of "α"-cholestenyl acetate had m. p. 94—95°, and was not depressed on admixture with the hydrogenation product. To confirm the composition of the latter 37 mg., dissolved in 5 ml. of carbon tetrachloride and 3 ml. of acetic anhydride, were treated at 10° with 5 drops of concentrated sulphuric acid according to Anderson and Nabenhauer's method (*J. Amer. Chem. Soc.*, 1924, **46**, 1957). After 20 minutes the mixture was diluted with water and worked up in the usual way. Chromatography over alumina, benzene being used as eluant, gave 28 mg. of cholestanyl acetate, m. p. 103—104° not depressed on admixture with authentic cholestanyl acetate (m. p. 107—108°).

Cholesta-7 : 9(11)-*diene*-3β : 6α-*diol*.—After separation of the cholesta-7 : 9(11)-diene-3β : 6β-diol as reported above the ethereal mother-liquors were evaporated *in vacuo* and the residue benzoylated (benzoyl chloride–pyridine overnight at room temperature). Working up in the usual way afforded *cholesta*-7 : 9(11)-*diene*-3β : 6α-*diol dibenzoate* which, recrystallised from chloroform–methanol, had m. p. 184—185°, $[\alpha]_D$ +144° (*c*, 2·58) (Found : C, 80·1; H, 8·1. $C_{41}H_{52}O_4$ requires C, 80·8; H, 8·6%).

Alkaline hydrolysis of this furnished cholesta-7 : 9(11)-diene-3β : 6α-diol; recrystallised from methanol, this had m. p. 194—195° (decomp.), $[\alpha]_D$ +94° (*c*, 0·55). Acetylation as above then gave the *diacetate*, m. p. 126—127° (from methanol), $[\alpha]_D$ +126° (*c*, 1·15), $\lambda_{max.}$ 242 mμ., $\varepsilon_{max.}$ 19,500 (Found : C, 76·5; H, 9·5. $C_{31}H_{46}O_4$ requires C, 76·8; H, 10·0%).

Windaus, Riemann, Rüggeberg, and Zühlsdorff (*Annalen*, 1942, **552**, 142) recorded m. p. 196° for this diol and isolated it as the bis-3 : 5-dinitrobenzoate. They did not characterise it further.

Cholest-8(14)-*ene*-3β : 6α-*diol*.—Cholesta-7 : 9(11)-diene-3β : 6α-diol dibenzoate (250 mg.) in 20 ml. of ether–AnalaR acetic acid was hydrogenated overnight over platinum. After working up in the usual way the product, once recrystallised from ethyl acetate–methanol, had m. p. 120—121°, $[\alpha]_D$ +25° (*c*, 2·75), +25° (*c*, 0·83). It was not investigated further but was hydrolysed in the usual way and the hydrolysis product acetylated (as above). The product was then chromatographed over alumina (6 fractions), to give *cholest*-8(14)-*ene*-3β : 6α-*diol diacetate*, eluted with 5% benzene–light petroleum; recrystallised from methanol, this had m. p. 141—142°, $[\alpha]_D$ +28° (*c*, 1·44) (Found : C, 76·5; H, 10·4. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·4%). It gave a yellow colour with tetranitromethane and showed $\lambda_{max.}$ 205 mμ., $\varepsilon_{max.}$ 8200, ε_{215} 4500. These absorption values are characteristic, within the limited range of possible double-bond positions, of olefinic unsaturation at 8(14) (private communication from Dr. H. B. Henbest, Manchester). The calculated rotation for cholest-8(14)-ene-3β : 6α-diol diacetate, based on $[\alpha]_D$ +38° for cholestane-3β : 6α-diol diacetate (see below) and the standard tables of Barton and Klyne (*Chem. and Ind.*, 1948, 755), is $[\alpha]_D$ +34°, in satisfactory agreement with the value found. The diacetate was also prepared by similar hydrogenation of cholesta-7 : 9(11)-diene-3β : 6α-diol diacetate.

Alkaline hydrolysis of cholest-8(14)-ene-3β : 6α-diol diacetate then gave the corresponding *diol* which, recrystallised from light petroleum, had m. p. 179—180°, $[\alpha]_D$ +24° (*c*, 1·43) (Found : C, 78·3; H, 11·3. $C_{27}H_{44}O_2,0{\cdot}5H_2O$ requires C, 78·7; H, 11·6%).

Benzoylation of the diol as above afforded the *dibenzoate*, m. p. 181—182° (from ethyl acetate–methanol), $[\alpha]_D$ +51° (*c*, 2·80) (Found : C, 81·7, 79·8; H, 8·5, 8·5. $C_{41}H_{54}O_4$ requires C, 80·6; H, 8·9%).

Hydrogenation of Cholest-8(14)-*ene*-3β : 6α-*diol Diacetate*.—(a) *Hydrogenation in acetic acid containing hydrogen chloride*. Cholest-8(14)-ene-3β : 6α-diol diacetate (25 mg.) in "AnalaR" acetic acid (20 ml.) containing a little hydrogen chloride gas was hydrogenated overnight in presence of a platinum catalyst. Working up in the usual way afforded cholestanyl acetate, m. p. 105—107°, $[\alpha]_D$ +16° (*c*, 0·68) in 0·5 dm. tube, on recrystallisation from methanol, undepressed in m. p. on admixture with an authentic specimen.

(b) *Hydrogenation after rearrangement*. Cholest-8(14)-ene-3β : 6α-diol diacetate (70 mg.), dissolved in chloroform (10 ml.), was treated with a stream of dry hydrogen chloride at room temperature for 30 minutes. After removal of the solvent *in vacuo* the residue was hydrogenated overnight in "AnalaR" acetic acid (20 ml.). The product was worked up in the usual way and the hydrogen chloride treatment and subsequent hydrogenation repeated twice more. If it is assumed that the equilibrium established by hydrogen chloride leads to equal amounts of 8(14)- and 14(15)-isomers (cf. Barton, Cox, and Holness, *J.*, 1949, 1771), then the repetitions of rearrangement and hydrogenation should have led to 87·5% of the required saturated product. To destroy any remaining unsaturated material the final hydrogenation

product was treated with chromium trioxide (30 mg.) in " AnalaR " acetic acid (10 ml.) on the water-bath for 30 minutes. After working up in the usual way the product was chromatographed over alumina (3 fractions), to give *cholestane*-3β : 6α-*diol diacetate*, eluted with 80% benzene–light petroleum and recrystallised from methanol; this had m. p. 55—56°, $[\alpha]_D$ +37° (*c*, 1·02), and gave no colour with tetranitromethane (Found: C, 76·1; H, 10·95. $C_{31}H_{52}O_4$ requires C, 76·2; H, 10·7%). The m. p. of this diacetate is very much lower than that (m. p. 107—108°, $[\alpha]_D$ +39°) recorded by Plattner and Lang (*Helv. Chim. Acta*, 1944, **27**, 1872). It appears that the diacetate prepared by us is a lower-melting form, for authentic specimens of cholestane-3β : 6α-diol diacetate were prepared (i) from cholestan-6-on-3β-yl acetate and (ii) from cholestane-3 : 6-dione, by reduction with sodium and *n*-propanol, followed by acetylation in pyridine overnight with acetic anhydride. In each case the crystal form (plates from methanol) was the same as that of the hydrogenation product and the m. p. (54—55°) and rotation {$[\alpha]_D$ +39° (*c*, 2·45)} were both in agreement. There was no depression in m. p. on admixture. To confirm the identity the hydrogenation product was hydrolysed to cholestane-3β : 6α-diol, m. p. 203—204° (from methanol) alone or mixed with authentic cholestane-3β : 6α-diol, m. p. 209—210°, prepared by alkaline hydrolysis of the authentic diacetate (see above). The m. p. of the diol is in agreement with that (m. p. 213—215°, corr.) recorded by Plattner and Lang (*loc. cit.*).

Reduction of Diphenylmercury by Lithium Aluminium Hydride.—Diphenylmercury (3·5 g.) in ether was treated with an excess of lithium aluminium hydride. Mercury immediately separated. After 0·5 hour's refluxing the solution was worked up and the mercury removed by filtration. Weight of mercury = 1·93 g. (Calc., 1·95 g.). The ethereal solution was washed with dilute sulphuric acid and water, and dried (Na_2SO_4). The ether was distilled off through a Vigreux column; the residue, recrystallised by freezing, had f. p. +2° undepressed by dry " AnalaR " benzene of f. p. +4·5°.

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BIRKBECK COLLEGE, LONDON, W.C.1. [*Received, May 11th*, 1951.]

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528. *Synthesis of* 1 : 3-*Dihydroxy*[2-^{14}C]*acetone.*

By H. R. V. ARNSTEIN and RONALD BENTLEY.

The synthesis of dihydroxyacetone described by Piloty (*Ber.*, 1897, **30**, 3161) has been improved and applied to the synthesis of dihydroxyacetone labelled with radioactive carbon. A new synthesis of dihydroxyacetone by acid decomposition of the sodium salt of 2-nitropropane-1 : 3-diol has been demonstrated in tracer experiments. This provides a rapid synthesis of labelled dihydroxyacetone when some dilution of radioactivity can be tolerated.

A SYNTHESIS of dihydroxyacetone labelled with ^{14}C was desired for investigations of carbohydrate metabolism. Since [^{14}C]formaldehyde (Arnstein, *Nature*, 1949, **164**, 361) and nitro-[^{14}C]methane (Sowden, *J. Biol. Chem.*, 1949, **180**, 55) had previously been prepared, the following synthesis of dihydroxyacetone (Piloty and Ruff, *Ber.*, 1897, **30**, 1656; Piloty, *ibid.*, p. 3161) appeared to be most useful :

$$3CH_2O + CH_3{\cdot}NO_2 \xrightarrow{\text{Alkali}} \underset{\text{(I.)}}{(HO{\cdot}CH_2)_3C{\cdot}NO_2} \xrightarrow{Na\text{-}Hg} \underset{\text{(II.)}}{(HO{\cdot}CH_2)_3C{\cdot}NH{\cdot}OH}$$

$$\xrightarrow{HgO} \underset{\text{(III.)}}{(HO{\cdot}CH_2)_3C{:}N{\cdot}OH} \xrightarrow{Br_2\text{-}H_2O} \underset{\text{(IV.)}}{(HO{\cdot}CH_2)_3CO}$$

With use of $^{14}CH_2O$ and $^{13}CH_3{\cdot}NO_2$ the product would be 1 : 3-dihydroxy[1 : 3-$^{14}C_2$]acetone, $(HO{\cdot}{}^{14}CH_2)_2CO$; with $^{14}CH_3{\cdot}NO_2$ and $^{13}CH_2O$, the 2-labelled compound would result. Further, by use of $^{14}CH_2O$ and $^{13}CH_3{\cdot}NO_2$, double labelling could be achieved.

Nitromethane may be condensed with aqueous formaldehyde solution, or with paraformaldehyde in organic solvents in presence of alkaline catalysts. The melting point of the product, trishydroxymethylnitromethane (I), has been variously reported as 144° to 158—162° (Henry, *Compt. rend.*, 1895, **121**, 210; Böeseken, *Rec. Trav. chim.*, 1915, **34**, 110; Schmidt and Wilkendorf, *Ber.*, 1919, **52**, 392; Kleinfeller, *ibid.*, 1929, **62**, 1584; Gorski and Makarov,

ibid., 1934, **67**, *B*, 996; de Maury, *Bull. Soc. chim.*, 1944, **11**, 281; Cason and Prout, *J. Amer. Chem. Soc.*, 1949, **71**, 1218). Using either aqueous or organic solutions we frequently obtained material melting as low as 130° which nevertheless gave good analytical results. The melting point was unchanged on crystallisation from ethyl acetate–chloroform, but recrystallisation from nitromethane (Cason and Prout, *loc. cit.*) gave white needles, m. p. 162°. It seemed likely that the variable melting point was caused by contamination with traces of the alkaline catalyst, especially since Gorski and Makarov (*loc. cit.*) had shown that alkali causes reversible decomposition. We found that if traces of inorganic material were removed by chromatography on alumina or treatment with ion-exchange resins, the melting point was raised to 176°.

Although Piloty (*loc. cit.*) and later Hersant and Linnell (*Quart. J. Pharm.*, 1931, **4**, 76) reduced (I) to the corresponding hydroxylamine (II) by sodium amalgam, we could not duplicate their yields (70 and 55%, respectively) (cf. Cason and Prout, *loc. cit.*, who obtained a yield of only 32·5%). In a search for a more reliable method, catalytic hydrogenation was investigated. In the presence of palladium–barium sulphate in methanol, hydrogen was rapidly taken up, but only trishydroxymethylmethylamine could be isolated. In aqueous solution, with the same catalyst, and in presence of oxalic acid as a trapping agent (Wilkendorf and Trénel, *Ber.*, 1923, **56**, 619; Schmidt, Ascherl, and Mayer, *ibid.*, 1925, **58**, 2430), hydrogen absorption was very slow; precise control was impossible and the product was a mixture containing mainly trishydroxymethylmethylamine oxalate.

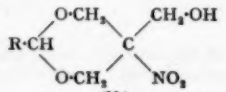
The use of aluminium amalgam in aqueous solution gave the hydroxylamine (II) in yields of up to 20%. An improved yield (65%) was obtained when (I), dissolved in tetrahydrofuran, was added to a stirred suspension of aluminium amalgam in ether.

Oxidation of (II) was carried out best in methanol. The oxime crystallised rather slowly and was always accompanied with a little oily material, which could not be removed by solvents; conversion into, and recovery from, the insoluble copper compound gave pure dihydroxyacetone oxime (III) in 65% yield. However, the crude oxime could be satisfactorily decomposed with bromine, so that this purification was normally omitted.

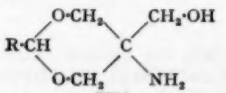
Owing to the solubility of dihydroxyacetone in many organic solvents, its isolation, particularly on the small scale used in this work, was attended with some loss. In most cases, the crystalline material was washed out with *n*-butanol; washings deposited further crops on storage. The yield of crystalline dihydroxyacetone obtained from the hydroxylamine (II) was 27%; the overall yield from nitromethane was 14·2% (cf. Hersant and Linnell, *loc. cit.*, 18·2% by Piloty's method).

The whole series of reactions was carried out with $^{14}CH_3{\cdot}NO_2$; after as much dihydroxyacetone as possible had crystallised, the residual mother-liquor was evaporated and treated with inactive "carrier" dihydroxyacetone. A further quantity of less active material was thus obtained, and the overall radiochemical yield from nitromethane was 21·8%.

Several alternative routes to dihydroxyacetone or glycerol (which can be converted into dihydroxyacetone) were also investigated. 2-Nitropropane-1 : 3-diol, readily obtainable from trishydroxymethylnitromethane, yields 2-aminopropane-1 : 3-diol on reduction (Schmidt and Wilkendorf, *loc. cit.*). The amine hydrochloride reacted smoothly with sodium or barium nitrite at a slightly acid pH, 80% of the theoretical volume of nitrogen being evolved in 2—3 hours. The crude product was an oil giving a strongly positive acraldehyde test when heated with sodium hydrogen sulphate. However, no significant amount of glycerol or its benzoate could be isolated. In general, deamination of amino-ketones or -alcohols appears to give poor yields and mixed products (*e.g.*, Chiari, *Monatsh.*, 1898, **19**, 578; Pictet and Barbier, *Helv. Chim. Acta*, 1921, **4**, 925; Kalischer, *Ber.*, 1895, **28**, 1521; Neuberg and Kansky, *Biochem. Z.*, 1909, **20**, 460) and this approach was therefore abandoned.



(V.)



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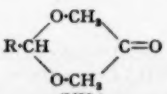


(VII.)

Periodate oxidation of the acetal (VI) of trishydroxymethylmethylamine to a cyclic acetal (VII) of dihydroxyacetone was considered possible, and such a protected compound might be more readily isolated. Trishydroxymethylnitromethane was converted into the acetal (V; R = Pr^n) by reaction with *n*-butaldehyde (Scattergood and MacLean, *J. Amer. Chem.*

Soc., **1949**, **71**, 4153) and was slowly reduced by hydrogen in presence of Adams's catalyst. The product (VI; R = Pr^n) was characterised as the crystalline benzoate, m. p. 113—115°; reaction with *p*-nitrobenzoyl chloride afforded only an unidentified, halogen-containing product, m. p. 71—72°. When an aqueous solution of the amine (VI; R = Pr^n) reacted with periodic acid, large quantities of iodine were liberated, but the crude product gave only a poor yield of heterogeneous 2 : 4-dinitrophenylhydrazones.

A second successful synthesis of dihydroxyacetone, involving only three stages from nitromethane, was found in the action of strong acid on the sodium salt of 2-nitropropane-1 : 3-diol (VIII), which salt was readily obtained by the action of sodium methoxide on (I) :

$$\underset{\text{(I.)}}{(HO{\cdot}CH_2)_2C{\cdot}NO_2} \xrightarrow{NaOMe} \underset{\text{(VIII.)}}{(HO{\cdot}CH_2)_2C{:}NO_2^-Na^+} \xrightarrow{H_2SO_4} \underset{\text{(IV.)}}{(HO{\cdot}CH_2)_2CO}$$

That strong mineral acid might decompose the sodium salt in this way was suggested by the fact that to obtain 2-nitropropane-1 : 3-diol it was best to acidify (VIII) with salicylic acid in boiling ether (Den Otter, *Rec. Trav. chim.*, **1938**, **57**, 13). Further, the synthesis was analogous to the conversion of nitro-deoxy-sugars (IX; R = H) into the corresponding aldoses (XI; R = H), and of compounds (IX; R = $CH_2{\cdot}OH$) into ketoses (XI; R = $CH_2{\cdot}OH$) (Sowden and Fischer, *J. Amer. Chem. Soc.*, 1944, **66**, 1312; 1945, **67**, 1713; 1946, **68**, 1511; 1947, **69**, 1048, 1963; Sowden, *ibid.*, 1950, **72**, 3325) :

$$\underset{\text{(IX.)}}{\begin{matrix}R{\cdot}CH{\cdot}NO_2\\ [CH{\cdot}OH]_n\\ CH_2{\cdot}OH\end{matrix}} \longrightarrow \underset{\text{(X.)}}{\begin{matrix}R{\cdot}C{=}NO_2^-Na^+\\ [CH{\cdot}OH]_n\\ CH_2{\cdot}OH\end{matrix}} \longrightarrow \underset{\text{(XI.)}}{\begin{matrix}R{\cdot}C{=}O\\ [CH{\cdot}OH]_n\\ CH_2{\cdot}OH\end{matrix}}$$

In many preliminary experiments, an aqueous solution of (VIII) was added to concentrated sulphuric acid under conditions used by Sowden (*loc. cit.*) in the synthesis of [1-^{14}C]glucose from (IX; R = H, *n* = 4). Removal of the excess of sulphuric acid by an aqueous suspension of calcium carbonate gave a solution which strongly reduced Fehling's solution in the cold. After treatment with ion-exchange resins and evaporation to dryness *in vacuo*, such solutions yielded viscous oils, which partly crystallised on seeding and prolonged storage. That dihydroxyacetone was, in fact, formed under these conditions was demonstrated as follows. $^{14}CH_2{\cdot}NO_2$ and formaldehyde were condensed and treated with sodium methoxide without purification of the intermediate (I). The radioactive sodium salt (VIII) was decomposed with acid; after de-ionisation, carrier dihydroxyacetone was added and the recovered oil crystallised on storage. By washing with alcohol 60% of the quantity of carrier added was recovered. Its radioactivity was unchanged on recrystallisation, and various derivatives were prepared and shown to have the same specific radioactivity. From the radioactivity of the isolated dihydroxyacetone, it was calculated that about 30% of the sodium salt had been converted into dihydroxyacetone. Where some dilution with carrier material can be tolerated, this method provides a rapid and convenient synthesis of labelled dihydroxyacetone. It is most convenient to carry out the initial condensation of $^{14}CH_2{\cdot}NO_2$ with paraformaldehyde in methanol; this solution of (I) is added to sodium methoxide in methanol, whereupon the sodium salt (VIII) is precipitated in nearly quantitative yield.

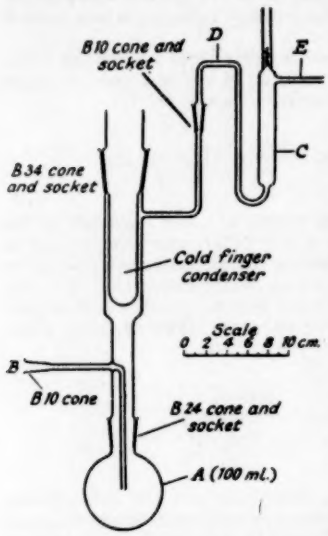
Experimental.

(M. p.s are uncorrected. Microanalyses by Drs. Weiler and Strauss, Oxford.)

Radioactivity Determinations.—The measurements were carried out on " infinite-thickness " samples, *i.e.*, 25 mg. or more of material/sq. cm., mounted on 1-sq.-cm. " Polythene " discs, as described by Popják (*Biochem. J.*, 1950, **46**, 560). Samples were counted with a helium-filled bell-shaped Geiger–Müller counter which had a thin mica window. The " background " of the instrument was 8—10 counts/min. and a sample which contained 10^{-3} μc. of ^{14}C/mg. of substance gave approx. 1100 counts/min. when counted as described above.

Preparation of Dihydroxyacetone by Piloty's Method.—(i) *Nitro*[^{14}C]*methane* (cf. Sowden, *loc. cit.*). $^{14}CH_2{\cdot}I$ ($26{\cdot}1 \times 10^{-3}$ μc./mg.; obtained from the Radiochemical Centre, Amersham) (6·1 g.) was distilled, with the aid of a vacuum manifold, into the reaction vessel (see figure). Flask *A* contained a mixture of silver nitrite (12·0 g.) suspended in non-radioactive nitromethane (13·4 g.) and was frozen in liquid air. The vessel was disconnected from the vacuum manifold and stoppered at *B*, and the contents of *A* were allowed to come to room temperature, with the cold finger containing solid carbon

dioxide in carbon tetrachloride. After an initial exothermic reaction, the contents were warmed at 85—90° for 1½ hours. The solid carbon dioxide mixture was then replaced by an ice–salt freezing mixture, and the methyl nitrite also produced was blown out in a stream of dry air (introduced at *B*) and condensed in the break-seal tube (*C*) which was surrounded with liquid air. After being sealed at *D* and *E*, this tube was removed, and the flask and condenser were again connected to the vacuum manifold. The nitromethane was then distilled in vacuum in a closed circuit into a weighed flask cooled in liquid air. The yield was 14·64 g.



To determine the radiochemical yield, ω-nitro[ω-^{14}C]styrene was prepared. To a solution of the above nitromethane (0·6 g.) and redistilled benzaldehyde (1·06 g.) in methanol (2 ml.), cooled in ice and stirred, was added slowly sodium hydroxide (0·42 g.) in ice-water (1·5 ml.). At this point more methanol (3 ml.) was added to thin the mixture. Water (8 ml.) was added after a few minutes, and the solution added dropwise to concentrated hydrochloric acid (2 ml.) and water (3 ml.). The yellow oil which formed was separated by decantation of the aqueous supernatant liquor; it was washed with a little water, and crystallised by addition of ethanol. The pale yellow crystals, recrystallised from ethanol, had m. p. 56°. Specific radioactivity was $3·29 \times 10^{-3}$ μc./mg. Therefore, the specific radioactivity of nitromethane = $8·03 \times 10^{-3}$ μc./mg., and hence the radiochemical yield of $^{14}CH_3{\cdot}NO_2$ from $^{14}CH_3I$ = 73·6%.

(ii) *Trishydroxymethylnitromethane* (I). (a) *Preparation from* $^{14}CH_3{\cdot}NO_2$. Nitro[^{14}C]methane (4·67 g.; $8·03 \times 10^{-3}$ μc./mg.), formaldehyde solution (40%; 17·50 g.), and anhydrous potassium carbonate (50 mg.) were mixed; after a short lag period the temperature rose to 50°, whereupon the flask was immersed in cold water to moderate the reaction. After 5 minutes, water (50 ml.) was added and the solution passed in succession through columns of Amberlite IR-100 (in the hydrogen cycle, 4·5 cm.^{2} × 40 cm.) and Deacidite E (4·5 cm.^{2} × 40 cm.). The columns were washed with water, a total of 2 l. of eluate being collected. This solution was evaporated in vacuum (bath-temp., < 60°) and the crystalline residue dissolved in warm ethyl acetate (100 ml.). This solution was evaporated in vacuum, and the white crystalline residue washed out with chloroform, to yield 8·35 g., m. p. 162—165° (a second crop, 1·25 g., was obtained on concentrating the chloroform washings; total yield, 83·5%). The specific radioactivity was $3·06 \times 10^{-3}$ μc./mg. A portion was recrystallised from nitromethane, forming long white needles, m. p. 176°, specific radioactivity $3·04 \times 10^{-3}$ μc./mg.

(b) *Purification of material of low m. p. by chromatography.* Trishydroxymethylnitromethane, when prepared according to the method of Schmidt and Wilkendorf, or of Henry (directions of Hersant and Linnell), usually had m. p. 129—131° (Found: C, 32·15; H, 5·1; N, 9·5. Calc. for $C_4H_9O_5N$: C, 31·8; H, 6·0; N, 9·3%). A solution of this material (1·0 g.) in methanol (5 ml.) was passed through a column of alumina (Hopkin and Williams; 2 cm.^{2} × 10 cm.); methanol was used as eluant, and 5 ml. fractions were collected. The pure compound (0·81 g.; m. p. 169—172°) was eluted in the second fraction.

(c) *Purification by ion-exchange resins.* Trishydroxymethylnitromethane (m. p. 129—131°; 51 g.) in water (500 ml.) was passed through columns of Amberlite IR-100 (5 cm.^{2} × 50 cm.) and Deacidite E (5 cm.^{2} × 50 cm.); washing was carried out with water, a total of 3 l. being collected. After evaporation to dryness *in vacuo* (bath-temp., < 60°) the residue was dissolved in warm ethyl acetate (150 ml.). The warm solution was dried (Na_2SO_4), filtered, and treated with chloroform until crystallisation took place. The white crystals obtained in two crops had m. p. 167—169°, the yield being 44 g.

(iii) *Trishydroxymethyl*[^{14}C]*methylhydroxylamine* (II). Trishydroxymethyl[^{14}C]nitromethane (1·0044 g.; specific activity, $3·06 \times 10^{-3}$ μc./mg. plus 1·9325 g. of inactive material; calculated specific activity, $1·05 \times 10^{-3}$ μc./mg.) in tetrahydrofuran (25 ml.) was added to aluminium amalgam (prepared as described by Vogel, *J.*, 1927, 594, from 1·2 g. of aluminium foil) under dry ether (75 ml.). Water (2 ml.) was added to the stirred mixture, which was warmed to boiling. The bath was removed, and the ether continued to reflux during the next 15 minutes. More water (2 ml.) was added and, after further warming, the mixture was stirred at room temperature for 45 minutes. The alumina precipitate was filtered off and washed with fifteen 40-ml. portions of water. The centrifuged washings were combined and evaporated to dryness *in vacuo* (bath-temp., < 55°). The crystalline residue was washed out with dry ether containing a little ethanol, to yield 1·675 g. (63·5%) of a white crystalline powder, m. p. 136—142°, specific activity, $1·10 \times 10^{-3}$ μc./mg.

(iv) *Dihydroxy*[2-^{14}C]*acetone oxime* (III). Trishydroxymethyl[^{14}C]methylhydroxylamine (1·0 g.; specific activity, $1·10 \times 10^{-3}$ μc./mg.), suspended in ethanol (20 ml.), was warmed at 65°; yellow mercuric oxide, freshly prepared from mercuric chloride (10 g.) and suspended in ethanol to form a thick paste, was added, with stirring, in portions. Initially a rapid blackening took place, and when the mixture had a pronounced yellow colour addition of mercuric oxide was stopped. The mixture was stirred for 2 hours at 60—65° and then filtered, and the residue washed with ethanol. The filtrate and washings, evaporated *in vacuo* (bath-temp., 40°), yielded an oil, which crystallised slowly. (This residue initially had a pronounced odour of formaldehyde.) A test portion did not reduce Fehling's solution in the cold. In later experiments, methanol was found to be a better solvent for this oxidation.

(v) *Dihydroxy*[2-^{14}C]*acetone* (IV). The above oxime, in water (8·0 ml.), was stirred during dropwise addition of bromine (0·5 ml.). Initially, the bromine was decolorised slowly, and there was a slow rise of temperature to 29°. After 10 minutes all the bromine had been added and an excess was present; the mixture was stirred for a further 1½ hours. At this time the solution was colourless. Hydrobromic acid was neutralised by addition of excess of lead carbonate, and the insoluble lead salts were filtered off. The filtrate and aqueous washings were passed in succession through columns of Amberlite IR-100 (H) (2 cm.^{2} × 25 cm.) and of Deacidite E (2 cm.^{2} × 30 cm.); the columns were washed with water, a total eluate of 2 l. being collected. After evaporation to dryness *in vacuo* (bath-temp., <40°) a yellow oil remained. It was dissolved in ethanol (5 ml.) and treated with ether (50 ml.). A white flocculent material was precipitated and soon coagulated to an oil. The turbid supernatant solution was decanted through a filter, and the filtrate evaporated to dryness *in vacuo* (bath-temp., <35°). A straw-coloured oil remained which was re-treated with ethanol (3 ml.) and ether (30 ml.). A little charcoal was added to the turbid solution, which was then filtered and evaporated to an oil as before. This oil crystallised quickly on seeding; after being kept overnight in a vacuum-desiccator with phosphoric oxide it was mixed with *n*-butanol (3 ml.) and filtered, and the residue washed with a little *n*-butanol–ether (1 : 2). There were obtained 105 mg. of a white crystalline powder, m. p. 76—78° (microblock; rapid heating), specific activity 1·62 × 10^{-3} μc./mg. On storage, the butanol filtrate deposited further crops: 40 mg., of specific activity 1·53 × 10^{-3} μc./mg.; 32 mg., of specific activity 1·47 × 10^{-3} μc./mg. The total yield was 177 mg., a chemical yield from (II) of 27·2%. The butanol filtrate was dissolved in water (10 ml.), and inactive dihydroxyacetone added as carrier (1·0 g.). The solution was evaporated to dryness *in vacuo* (bath-temp., <35°) to yield an oil, which slowly crystallised. On treatment with *n*-butanol, 900 mg. of dihydroxyacetone were recovered, having specific activity 0·190 × 10^{-3} μc./mg. Hence, the total recovery of radioactivity = 449·5 × 10^{-3} μc., the radiochemical yield being 41·1%. The overall radiochemical yield of dihydroxyacetone from nitromethane = 21·8%.

Preparation of Dihydroxyacetone from the Sodium Salt of 2-Nitro[2-^{14}C]*propane*-1 : 3-*diol* (VIII).—(i) *Sodium salt* (VIII). $^{14}CH_2{\cdot}NO_2$ (1·1102 g., specific activity 8·03 × 10^{-3} μc./mg.; plus inactive material, 0·6078 g.; calculated specific activity, 5·19 × 10^{-3} μc./mg.) was mixed with formaldehyde solution (40%; 6·450 g.) and potassium carbonate (50 mg.). A vigorous reaction took place after a few minutes' shaking, and at 50° the flask was cooled with water. When the mixture had cooled to room temperature the solution was evaporated to dryness *in vacuo* (bath-temp., <50°). The crystalline residue was dissolved in methanol (50 ml.) and added to a solution of sodium (1 g.) in methanol (50 ml.). A white precipitate formed almost at once; it was filtered off after 1 hour at 0° to yield material [4·773 g. (91%)] containing 2 molecules of methanol of crystallisation.

(ii) *Dihydroxy*[2-^{14}C]*acetone*. The above sodium salt (4·773 g.) in water (35 ml.) was added slowly with stirring to a mixture of concentrated sulphuric acid (3 ml.) and water (5 ml.) cooled in an ice-bath (average internal temperature during addition, 5—10°). As each portion was added a blue-green colour developed and there was some gas evolution. When addition was complete (15 minutes) the solution was stirred for 10 minutes at room temperature. It was then added to a stirred suspension of calcium carbonate (10 g.) in water (50 ml.); when neutralisation was complete, the mixture was filtered and the solid washed with water (the filtrate reduced Fehling's solution in the cold). Inactive dihydroxyacetone (1·99 g.) was added to the filtrate and washings, which were then passed in succession through columns of Amberlite IR-100 (H) (5 cm.^{2} × 50 cm.) and of Deacidite E (5 cm.^{2} × 50 cm.). The columns were washed with water, a total of 2 l. being collected; this eluate was evaporated to dryness *in vacuo* (bath-temp., <40°). A brownish oil remained, which was dissolved in ethanol (20 ml.) and treated with ether (150 ml.). A flocculent precipitate formed; charcoal was added, and, after filtration, the clear solution was evaporated *in vacuo* (bath-temp., 30°). A pale yellow oil was obtained and was treated again with ethanol, ether, and charcoal. Removal of solvent as before gave a pale oil; this was seeded and kept in a vacuum-desiccator for 3 days. The crystalline product was filtered off after addition of *n*-butanol (5 ml.), to yield 1·025 g. of white crystals, m. p. 85—89° (slow heating in an oil-bath), specific activity, 0·877 × 10^{-3} μc./mg. The radiochemical yield from nitromethane = 10·1%.

In a second experiment, trishydroxymethylnitro[^{14}C]methane (3·0 g.; specific activity, 1·17 × 10^{-3} μc./mg.) in methanol (30 ml.) was treated with sodium (0·67 g.) in methanol (30 ml.). The sodium salt was filtered off and dissolved in ice-water (50 ml.), and this solution added to concentrated sulphuric acid (3 ml.) and water (4 ml.) in an ice-bath. The solution was worked up as described previously, but carrier dihydroxyacetone (2·03 g.) in water (10 ml.) was added only after the ethanol–ether precipitation. The dihydroxyacetone, isolated as usual (yield, 1·11 g.), had m. p. 85—88° (slow heating in oil-bath), specific activity, 0·340 × 10^{-3} μc./mg. A second crop (0·100 g.), of specific activity 0·342 × 10^{-3} μc./mg., was obtained from the alcoholic filtrate (ice-cold ethanol was used as filtering agent in this experiment). The charcoal used in treating the ethanol–ether solutions was suspended in water (20 ml.) containing inactive dihydroxyacetone (1·0 g.). Recovery of the material gave 0·70 g. of dihydroxyacetone, specific activity 0·077 × 10^{-3} μc./mg. [radiochemical yield from (I) = 13·3%].

For recrystallisation, 200 mg. of the first crop were dissolved in warm absolute alcohol (7·5 ml.), filtered, and treated with ether (25 ml.). The white crystals separating on cooling (24 mg.) had specific activity 0·342 × 10^{-3} μc./mg.

In order to establish the purity of dihydroxyacetone prepared in this way, three derivatives were prepared, by standard methods, from the material obtained at the first crop. The observed specific activities of these derivatives, and the calculated specific activities (based on the value 0·340 × 10^{-3} μc./mg. for dihydroxyacetone) are shown in the Table. The compounds had substantially the recorded m. p.s.

5-Hydroxymethyl-5-nitro-2-n-propyl-1 : 3-*dioxan* (V; R = Pr^n).—Trishydroxymethylnitromethane (10·5 g.), concentrated hydrochloric acid (5·6 ml.), water (6·7 ml.) and *n*-butaldehyde (5 g.) were shaken vigorously at room temperature for 40 hours. The solid, which had separated, and the supernatant

Observed and calculated specific radioactivities (10^{-3} μc./mg.) *of derivatives of dihydroxy*[2-^{14}C]*acetone*.

	Observed	Calc.[a]
Phenylosazone	0·112,[c] 0·112 [b]	0·114
2 : 4-Dinitrophenylhydrazone	0·115	0·113
Methylglyoxal disemicarbazone	0·159,[c] 0·165 [d]	0·164

[a] Original material. [b] Recrystallised. [c] First crop. [d] Second crop.
[e] Based on the value 0·340 × 10^{-3} μc./mg. for dihydroxyacetone.

solution were then extracted with ether, and the residue remaining on evaporation of the ether was recrystallised from *n*-hexane, yielding the acetal (5·3 g.), m. p. 99—101°.

5-Amino-5-hydroxymethyl-2-n-propyl-1 : 3-dioxan (VI; R = Pr^n).—A solution of the above compound (5·3 g.) in methanol (65 ml.) was hydrogenated at room temperature and atmospheric pressure in the presence of Adams's catalyst (150 mg.). The uptake of hydrogen was approx. 30 ml./hour but after 3 days the theoretical volume (1660 ml.) had been absorbed. The catalyst was removed by filtration, and the solvent evaporated *in vacuo*. The residual oil (4·46 g., 98%) was characterised as the crystalline *benzoate*, prepared by reaction with benzoyl chloride in pyridine. After sublimation in a high vacuum this had m. p. 113—115° (Found: C, 64·3; H, 7·95; N, 5·2. $C_{15}H_{21}O_4N$ requires C, 64·5; H, 7·6; N, 5·0%). Similar reaction of the amine with *p*-nitrobenzoyl chloride in pyridine afforded only an unidentified, halogen-containing substance, m. p. 71—72° (after sublimation *in vacuo*) (Found: C, 49·5, 49·2; H, 3·6, 3·3; N, 8·0; Cl, 18·6%).

A solution of the acetal (V; R = Pr^n) (5·7 g., 0·0278 mole) and oxalic acid (1·76 g., 0·014 mole) in methanol (50 ml.)–water (35 ml.) was hydrogenated with 5% palladium–barium sulphate. The uptake of hydrogen was 15 ml./hour; Adams's catalyst (300 mg.) was therefore added, and the hydrogenation continued at 90 atm. for 5 hours. The solution was filtered, and the filtrate concentrated to about 50 ml., whereupon crystalline starting material separated (2·7 g.) (m. p. and mixed m. p. 98—100°). The filtrate was extracted with ether and the aqueous layer was evaporated to dryness. The residue was crystallised from ethanol by addition of ether, giving the *hydrogen oxalate* (0·75 g.), m. p. 176—177°, of (VI; R = Pr^n) (Found: C, 45·0; H, 7·2; N, 5·6, 5·0. $C_{10}H_{19}O_7N$ requires C, 45·3; H, 7·2; N, 5·3%).

2-Nitropropane-1 : 3-diol (cf. Den Otter, *loc. cit.*).—Finely powdered sodium salt of 2-nitropropane-1 : 3-diol (salt prepared as previously described, 45·0 g.), suspended in dry ether (1 l.), was added to a boiling solution of salicylic acid (30 g.) in dry ether (800 ml.). Enough dry ether to bring the volume to 2·5 l. was then added and the solution was refluxed for 90 minutes. The solution was filtered and evaporated to dryness *in vacuo*, yielding an oil which crystallised after about 30 minutes. The product was recrystallised from 270 ml. of ethyl acetate–chloroform (1 : 1·7). The yield was 16 g., and the m. p. 55—57°.

2-Aminopropane-1 : 3-diol (Schmidt and Wilkendorf, *loc. cit.*).—A solution of 2-nitropropane-1 : 3-diol (13·5 g.) and oxalic acid dihydrate (7·0 g.) in water (75 ml.) was hydrogenated in presence of 5% palladium–barium sulphate. When hydrogen uptake was complete, the solution was filtered and the filtrate concentrated *in vacuo*. Addition of acetone yielded 2-aminopropane-1 : 3-diol oxalate (12 g.), m. p. 200°.

Reaction of 2-Aminopropane-1 : 3-diol with Nitrous Acid.—(*a*) 2-Aminopropane-1 : 3-diol oxalate (2·7 g., 0·01 mole) in water (20 ml.) was treated with barium chloride (2·1 g., 0·005 mole) in water (10 ml.). The precipitated barium oxalate was removed by centrifugation and to the supernatant solution of aminopropanediol hydrochloride was added a solution of sodium nitrite (3·0 g.) in water (10 ml.). There was a vigorous evolution of nitrogen (300 ml. in 3 hours). The solution was filtered and evaporated to dryness *in vacuo* (bath-temp., < 50°). The residue was extracted with dry methanol, and the solution was filtered and evaporated to dryness. This process was repeated twice more with methanol and once with methanol–ether (1 : 1). The final extract was evaporated and dried *in vacuo*, the yield being 1·46 g. This product gave a positive acraldehyde test. Repetition of this preparation on a larger scale gave a product which was more viscous than glycerol and could not be distilled *in vacuo* without extensive decomposition. Reaction of this material with benzoyl chloride in pyridine, a procedure which gives glycerol tribenzoate, m. p. 75°, in 75% yield, failed to give a solid benzoate.

(*b*) A solution of barium nitrite–barium chloride, prepared by shaking silver nitrite (1·9 g.) with a solution of barium chloride (dihydrate) (3·05 g., 0·0125 mole) for 10 minutes and filtration to remove silver chloride, was added, with cooling, to the aminopropanediol oxalate (1·68 g., 0·0125 mole) in water (25 ml.). The barium oxalate was removed by centrifugation and washed once with water. The combined supernatant solution began to evolve nitrogen; it was allowed to warm slowly. After 2 hours at room temperature it was heated for 10 minutes at 100°. The solution was then kept at room temperature for 16 hours, and evaporated *in vacuo* at 40°. The residue was extracted with methanol; the methanol was evaporated and the residue extracted with methanol–ether (1 : 1). This solution was filtered and evaporated. The oil was dried in a vacuum-desiccator. No glycerol tribenzoate was obtained by reaction of the product with benzoyl chloride in pyridine.

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529. *Molecular-orbital Calculations for Some Aromatic Hydrocarbons. Part I.*

By R. D. Brown.

Molecular-orbital calculations of π-electron densities, mobile bond orders, free valencies, resonance energies, and spectra are presented for the hydrocarbons *s*-indacene, *as*-indacene, pyracylene, and dibenzopyracylene. The results are used to predict the chemical properties of the molecules. Comparisons are drawn between the electronic structures of these four molecules and other related non-alternant hydrocarbons.

The observed spectrum of dibenzopyracylene agrees surprisingly well with that calculated by the simplest L.C.A.O. approximation.

In two earlier papers (Brown, *Trans. Faraday Soc.*, 1949, **45**, 296; 1950, **46**, 146) the results of molecular-orbital calculations were presented for four simple non-benzenoid hydrocarbons with a view to predict their chemical properties. In the present paper this study is extended to four additional non-benzenoid hydrocarbons. The results are of particular interest because one of the compounds considered has recently been synthesised (Clar, Stubbs, and Tucker, *Nature*, 1950, **166**, 1075) and so offers a further opportunity to test the reliability of the simplest molecular-orbital approximation, in particular when used to predict the chemistry of non-alternant hydrocarbons. So far the only study of the substitution in a non-alternant hydrocarbon is that of azulene (Anderson and Nelson, *J. Amer. Chem. Soc.*, 1950, **72**, 3824), which confirms some of the properties predicted by the M.O. approximation (Brown, *Trans. Faraday Soc.*, 1948, **44**, 984).

Method of Computation.—The usual L.C.A.O. approximation (Coulson and Longuet-Higgins, *Proc. Roy. Soc.*, 1947, *A*, **191**, 39) was employed; some complementary details of the method have been summarised elsewhere (Brown, *Austral. J. Sci. Res.*, 1949, *A*, **2**, 564). The Coulomb integrals of all atoms and the resonance integrals of all bonds were taken to be the same, no attempt being made to correct the former for the variation of the charges, q, from unity, or to correct the resonance integrals for variations in mobile bond order, p. The energy quantities were computed, the overlap integral, S, between adjacent atomic orbitals being assumed to be (i) zero, and (ii) 0·25. The symbolism employed to distinguish these two cases has already been described by the author (*loc. cit.*, 1950). The calculated values of p and q are independent of the value assumed for S (Löwdin, *J. Chem. Physics*, 1950, **18**, 365; Chirgwin and Coulson, *Proc. Roy. Soc.*, 1950, *A*, **201**, 196). For the sake of uniformity the free valency numbers, F, were computed by using $N_{max.} = 4{\cdot}680$ (see Brown, *Trans. Faraday Soc.*, 1949, **45**, 296), although theoretically $3 + \sqrt{3}$ is preferable.

The assumptions implied in the correlation of the various theoretically-defined M.O. quantites with chemical properties have been discussed (Brown, *loc. cit.*, 1950). Again, a high value of F has been taken to indicate a high homolytic reactivity although the precise significance of free valency is still *sub judice*.

An interesting case of accidental degeneracy occurs in both pyracylene and dibenzopyracylene. The degeneracy occurs in both molecules in the totally symmetric factor of the secular determinant. Consequently, care must be taken to select orthogonal solutions in calculating q and p. In the author's experience this behaviour is unique in such small molecules,* the only cases previously encountered being degeneracies among energy levels of different symmetry classes, when the solutions are of course necessarily orthogonal.

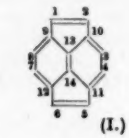
Nomenclature.—The hydrocarbon $C_{14}H_8$ (I) has not hitherto been prepared. Attempts were made to prepare the 1 : 2 : 5 : 6-tetrahydro-derivative by Fleischer and Wolff (*Ber.*, 1920, **53**, 925), who suggested the name pyracene for this reduced derivative. From analogy with acenaphthylene and acenaphthene the name *pyracylene* suggests itself for (I). The compound synthesised by Kloetzel and Chubb (*J. Amer. Chem. Soc.*, 1950, **72**, 150) could then be termed 1 : 2-dihydro-4 : 5-benzopyracylene instead of 1 : 2-dihydro*cyclo*penta[*cd*]fluoranthene, and the compound (II) could be termed 1 : 2-5 : 6-dibenzopyracylene in preference to bis*peri*phenylene-naphthalene.

Results.—The hydrocarbons studied were *s*-indacene (III),† *as*-indacene (IV), pyracylene (I),

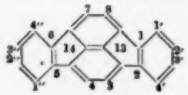
* It is, however, found in the higher polyphenyls and other large molecules.

† That is, the "Ring Index" indacenes, $C_{12}H_8$, and not the compounds $C_{12}H_{10}$ often called indacenes.

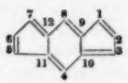
and 1 : 2-5 : 6-dibenzopyracylene (II). We first consider *s*-indacene. The resonance-energy data (see table) show that *s*-indacene is more stable than its *as*-isomer, a result which can be explained qualitatively in terms of the simplest "resonance" concepts. *s*-Indacene has two equivalent most stable contributing structures, (III*a*) and (III*b*), as does benzene. On the other hand, the two most stable contributing structures, (IV*a*) and (IV*b*), for *as*-indacene are



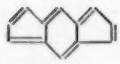
(I.)



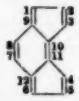
(II.)



(III*a*.)



(III*b*.)



(IV*a*.)

(IV*b*.)

(V.)

Resonance energies.

Hydrocarbon.	$R(-\beta)$.	$R'(-\gamma)$.	Hydrocarbon.	$R(-\beta)$.	$R'(-\gamma)$.
s-Indacene	4·2314	1·9757	1 : 2-5 : 6-Dibenzopyracylene	9·2842	4·5865
as-Indacene	3·8987	1·6912	Difulvyl	3·4680	1·5472
Pyracylene	5·4156	2·5578			

not equivalent. The mobile-order results (see below) indicate a greater contribution from (IV*b*). It is of interest in view of this to compare the resonance energy of difulvyl (V), which has for convenience been included in the above table. The increase in R' for indacene is $-0{\cdot}14\gamma$, considerably smaller than the increase ($-0{\cdot}29\gamma$) accompanying the analogous structural change from stilbene to phenanthrene.

The π-electron densities, q, the free valencies, F, and mobile orders, p, of *s*-indacene are listed in the following table. The data for q indicate a high electrophilic reactivity at position 2

s-Indacene.

Position.	q.	F.	Bond.	p.	Position.	q.	F.	Bond.	p.
1	0·952	0·480	1 : 2	0·658	4	0·776	0·490	4 : 10	0·595
2	1·109	0·363	9 : 1	0·542	9	1·106	0·075	9 : 10	0·468

(position 9, of course, does not carry a hydrogen to be substituted), while position 4 should be most susceptible to nucleophilic attack; methyl groups in this position would be expected to be relatively reactive with aldehydes for example. The free valencies point to homolytic attack at position 4 also. Comparison of the charge-density data with the corresponding results for pentalene (Brown, *loc. cit.*, 1949) reveals that the insertion of a benzene ring between the two five-membered rings leaves the relative charge distribution in the latter unaltered. The mobile orders show that the bonds are all relatively inert so that reaction with osmium tetroxide will not occur.

The corresponding results for *as*-indacene are tabulated below. For this molecule we should expect electrophilic substitution to occur at position 2 and nucleophilic reactivity to be greatest at position 7, as judged by the π-electron densities. The free valencies are all unusually

as-*Indacene.*

Position.	q.	F.	Bond.	p.	Position.	q.	F.	Bond.	p.
1	1·043	0·501	1 : 2	0·790	7	0·811	0·475	3 : 10	0·676
2	1·069	0·385	2 : 3	0·495	9	0·988	0·126	9 : 10	0·464
3	0·978	0·501	7 : 8	0·503	10	1·111	0·124	8 : 9	0·702
								10 : 11	0·416

high and indicate position 3 as the centre of radical attack. In this case the bond orders show a much greater variation and indicate a high reactivity for the 1 : 2 bond. The mobile order of

this bond (0·790) is similar to that of the reactive bonds in angular polycyclic hydrocarbons and so *as*-indacene might be expected to react with osmium tetroxide.

Comparison of these results with those for the interesting hydrocarbon fulvalene (Brown, *loc. cit.*, 1949; *Nature*, 1950, **165**, 566) shows that the addition of the ethylene bridge leaves the π-electron densities in the five-membered rings qualitatively unaltered, although there is a considerable rearrangement in the bond orders.

We now consider pyracylene (I), for which the results are given below. They indicate positions 1 and 3 as the sites of electrophilic and nucleophilic substitutions, and greatest homolytic reactivity at position 3. The mobile orders indicate a high reactivity for the 1 : 2-bond. For comparison, the positions in acenaphthylene corresponding to 1 and 3 in pyracylene have q values of 1·066 and 0·909, respectively, and the order of its 1 : 2-bond is 0·796 (the data for acenaphthylene given by Sandorfy, *Compt. rend.*, 1948, **227**, 198, would appear to contain a numerical error). This shows that the addition of the ethylene bridge to

Pyracylene.

Position.	q.	F.	Bond.	p.	Position.	q.	F.	Bond.	p.
1	1·042	0·444	1 : 2	0·830	9	0·996	0·106	9 : 1	0·406
3	0·917	0·417	3 : 4	0·610				10 : 3	0·652
								13 : 14	0·531
								9 : 13	0·516

acenaphthylene does not very greatly disturb the electronic structure of the molecule. Pyracylene may alternatively be considered to be derived from fulvalene by addition of two ethylene bridges; comparison with the data for fulvalene (*loc. cit.*) reveals great reorganisation of the π-electrons accompanying this structural change. Pyracylene differs from *as*-indacene by an ethylene bridge, and here again the π-electron densities and mobile orders of corresponding positions show a marked similarity in these two molecules.

1 : 2-5 : 6-*Dibenzopyracylene.*

Position.	q.	F.	Bond.	p.	Position.	q.	F.	Bond.	p.
1′	0·988	0·388	1′ : 2′	0·665	9	1·001	0·122	1 : 2	0·565
2′	1·003	0·359	2′ : 3′	0·656	14	1·050	0·104	1 : 9	0·385
3	0·958	0·395	3 : 4	0·631				9 : 13	0·520
1	1·026	0·103	1 : 1′	0·627				8 : 9	0·654
								13 : 14	0·536

Finally, we consider dibenzopyracylene. The dibenz-annelation is seen (table) to have the effect of making the π-densities more uniform. We should expect electrophilic attack at positions 2′ and nucleophilic substitution at position 3, but the values of q are so similar that the self-atom-polarisabilities may well be more important in determining the course of substitution reactions. The free valencies indicate high homolytic reactivity at position 1′. It will be noticed that the mobile orders are all lower than the benzene value of 0·667, so we should expect dibenzpyracylene to be inert to bond reagents such as osmium tetroxide. These predictions for dibenzopyracylene are of particular interest since the synthesis of this compound has recently been announced, and the ultra-violet absorption spectrum published (Clar, Stubbs, and Tucker, *loc. cit.*). The spectra of the compounds whose chemistry has just been considered are listed below. These results have been derived by the simplest L.C.A.O. treatment, including overlap, but not antisymmetrizing or allowing for configurational interaction. These latter refinements seem to be rather important in the case of small alternant hydrocarbons such as butadiene (Parr and Mulliken, *J. Chem. Physics*, 1950, **18**, 1338), benzene (Parr, Craig, and Ross, *ibid.*, p. 1561), and naphthalene (Jacobs, *Proc. Physical Soc.*, 1949, **62**, 710), and presumably the same applies to larger hydrocarbons, including non-alternants. However, such an elaborate treatment would be extremely tedious for molecules as large as those considered here, and there is some evidence (Pullman, *Compt. rend.*, 1949, **229**, 887) that the present semi-empirical approach is reasonably good for larger hydrocarbons.

There is little point in discussing the first three spectra apart from pointing out that the results for pyracylene will be particularly unreliable since there is an accidental degeneracy for two of the B_{2u} levels (see above). In this case configurational interaction will be of particular importance in determining the position of these levels relative to the others. In the case of dibenzopyracylene, however, the spectrum has already been observed experimentally and the observed band positions have been converted into transition energies for inclusion in the table.

Absorption spectra.

Hydrocarbon.	Transition.*	Transition energy, ev. Calc. †	Found.	Polarization.*
s-Indacene	$A_g - B_{3u}$	1·8	—	forbidden
	$A_g - B_{1u}$	1·8	—	z
	$A_g - B_{2u}$	3·0	—	y
as-Indacene	$A_1 - B_2$	0·2	—	y
	$A_1 - B_2$	2·0	—	y
	$A_1 - A_1$	2·6	—	z
Pyracylene	$A_g - B_{3u}$	1·3	—	forbidden
	$A_g - B_{1u}$	2·7 ‡	—	y
	$A_g - B_{1u}$	2·7	—	z
Dibenzopyracylene	$A_g - B_{3u}$	2·4	2·5	forbidden
	$A_g - B_{1u}$	2·5	3·0	z
	$A_g - B_{2u}$	3·4	4·3	y

* The yz plane has been taken to correspond to the molecular plane. Where there are two symmetry axes in the molecular plane the z axis is taken to be the longer dimension of the molecule.

† The transition energies have been converted into ev. by use of the conversion factor $-\gamma = 3{\cdot}4$ ev. which was employed by Brown (*loc. cit.*, 1950).

‡ In the approximation employed there is here an accidental degeneracy of two excited B_{3u} levels.

The agreement is quite good, the more so because the first transition has an intensity of the order of magnitude expected for a symmetry-forbidden transition, while the other two have the appearance of permitted transitions. A similar agreement with observation was found in the case of diphenylene (*Trans. Faraday Soc.*, 1950, **46**, 146). Such results suggest that the simple M.O. treatment of spectra may be reasonably satisfactory for larger molecules where it is impracticable to carry through the more complete treatment incorporating configurational interaction.

Appendix.

For convenience, the following data are added.

Energies (β) *of the occupied molecular orbitals.*

s-Indacene.		*as*-Indacene.		Pyracylene.		Dibenzopyracylene.	
2·4383	1·1386	2·4605	1	2·5616	1	2·6597	1
2	0·6180	1·9377	0·8323	2	1	2·3315	1
1·3028	0·6180	1·1442	0·3046	1·7321	0·4142	1·8549	1
				1		1·7746	0·6180
						1·5208	0·5798
						1·3028	

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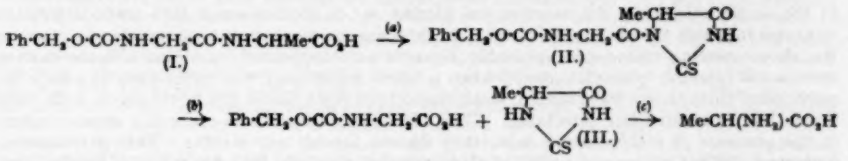
530. *The Stepwise Degradation of Peptides.*

By S. G. Waley and J. Watson.

A method for the elucidation of the amino-acid sequence in peptides by conversion of the residue bearing a terminal carboxyl group into a thiohydantoin (Schlack and Kumpf, *Z. physiol. Chem.*, 1926, **154**, 125) has been further investigated. By use of paper chromatography it has been found advantageous to identify the terminal residue as the amino-acid. Application of the method to insulin has revealed alanine as a terminal amino-acid.

Several methods for the stepwise degradation of peptides have been described recently which are concerned with the amino-acid bearing a terminal amino-group (Khorana, *Chem. and Ind.*, 1951, 129; Fox, *Adv. Protein Chem.*, 1945, **2**, 155; Edman, *Acta Chem. Scand.*, 1950, **4**, 283; Levy, *J.*, 1950, 404). Fewer methods, however, are available for the carboxyl end of the peptide chain (Fox, *loc. cit.*); the most promising utilises the formation of a thiohydantoin (Schlack and Kumpf, *loc. cit.*) : this method has been applied to glutathione (Nicolet, *J. Biol. Chem.*, 1930.

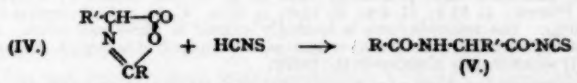
88, 389). The peptide is heated with acetic anhydride and ammonium thiocyanate, and the acyl-thiohydantoin, *e.g.*, (II), is then hydrolysed with dilute alkali.



Hitherto the terminal residue has been identified by isolation of the thiohydantoin, *e.g.*, (III); it is, however, more convenient to convert the thiohydantoin into the amino-acid, which can be purified and identified by paper chromatography. In this way, only small amounts of the peptide are needed, and, by Boissonas's method (*Helv. Chim. Acta*, 1950, **33**, 1975), the amount of amino-acid on the chromatogram can be determined.

Stage (a). Experiments carried out with carbobenzyloxyglycyl-DL-alanine (I) gave 1-carbobenzyloxylglycyl-5-methyl-2-thiohydantoin (II) in 73% yield under the conditions employed by Schlack and Kumpf (*loc. cit.*). Since proteins and peptides are not soluble in acetic anhydride alone, the cyclisation was investigated in various mixtures of acetic anhydride with formic acid, formamide, dimethylformamide, and dichloroacetic acid. Thiohydantoin formation occurred in each case, but the yields were low, except in acetic anhydride containing 20% of dichloroacetic acid.

An alternative method of thiohydantoin formation was also investigated. The formation of thiohydantoins by the action of acetic anhydride and ammonium thiocyanate on acylamino-acids proceeds through the oxazolone (IV), which probably reacts with thiocyanic acid to form the acyl *iso*thiocyanate (V) (Johnson and Scott, *J. Amer. Chem. Soc.*, 1913, **35**, 1136). If the



latter could be formed directly from the acylamino-acid, side reactions which may be occasioned by the labile oxazolone (IV) would be avoided. Therefore the action of benzoyl *iso*thiocyanate on the peptide (I) was examined (cf. the preparation of acid chlorides by use of benzoyl chloride; Brown, *J. Amer. Chem. Soc.*, 1938, **60**, 1325). Cyclisation only occurred under such vigorous conditions (heating the reactants in the absence of a solvent at 120°) that this modification does not seem suitable for use on peptides.

Stage (b). The hydrolytic fission of the 1-acyl-2-thiohydantoins was carried out by Schlack and Kumpf (*loc. cit.*) with N-alkali. We found that these conditions were unnecessarily vigorous: 1-acetyl-5-methyl-2-thiohydantoin lost the acetyl group in only a few minutes in 0·1N-alkali at room temperature, and in about an hour in 0·01N-alkali. The thiohydantoins are such weak acids [*e.g.*, (III), pK = 9·1; du Vigneaud and Melville, "The Chemistry of Penicillin," Princeton Univ. Press, 1949, p. 288] that they can be extracted from neutral solution with organic solvents.

Stage (c). The thiohydantoin (III) was converted into alanine by hydrobromic acid at 155° in 6 hours: the yield was 90% and was not changed by increasing the time of heating to 20 hours. All yields are based on the colorimetric estimation of alanine after paper chromatography by Boissonas's method (*loc. cit.*). The overall recovery of alanine from the acylthiohydantoin (II) in stages (*b*) and (*c*) was 84%.

Since the method worked smoothly with the simple peptide (I), we applied it to insulin. In the preliminary experiments reported here, the protein was not oxidised to break the linkages between the chains (Sanger, *Biochem. J.*, 1949, **44**, 126) but was acetylated to protect the amino- and hydroxy-groups and then heated with acetic anhydride and ammonium thiocyanate. After stages (*b*) and (*c*) had been carried through as described above, paper chromatography of the product (development with phenol) showed the presence of alanine and a trace of material with the same R_F as aspartic acid. Although no accurate measurement was made of the amount of alanine formed in this experiment, the yield was approximately 1 μg. from 1 mg. of insulin. Lens (*Biochim. et Biophys. Acta*, 1949, **3**, 367), using carboxy-peptidase, has shown that there may be one, two, or three terminal alanine residues with free carboxyl groups in the

insulin sub-unit of molecular weight 12,000.* Thus, at least 7·4 μg. of alanine should be obtained from 1 mg. of insulin.

Since the low yield might be partly attributed to the low solubility of the acetylated insulin in the acetic anhydride, the reaction was carried out in dichloroacetic acid–acetic anhydride mixtures in which the protein dissolved. In this case, however, as well as the alanine, more of the slower-moving amino-acid (probably aspartic acid) appeared, and also a faster-moving amino-acid (possibly phenylalanine); when a blank experiment was undertaken in which the ammonium thiocyanate was omitted, small amounts of these slower and faster amino-acids were found, but no alanine was detectable. Thus, although side reactions occur to a greater extent in the presence of dichloroacetic acid, they do not furnish any alanine. This modification, moreover, did not improve the yield of alanine, which was only 0·5 μg. per mg. of insulin. This result suggested that the losses might occur in the isolation of the thiohydantoin (III) from solutions containing insulin. This was confirmed when it was found that the recovery of (III) from a solution containing acetylated insulin was only 17%. There appears to be some interaction between the thiohydantoin and the protein which prevents the extraction of the thiohydantoin from neutral or acid solutions.

It is, of course, essential that any method for the stepwise degradation of polypeptides should give high yields in the removal of each residue. Although from our experiments with the simple peptide (I) the thiohydantoin method seemed promising, if the preliminary experiments on insulin described above are typical, there are considerable difficulties in its application to proteins. For the more limited objective of determining the residues bearing a terminal carboxyl group the method may be more useful.

Experimental.

1-Carbobenzyloxy-5-methyl-2-thiohydantoin (II).—(1) Carbobenzyloxyglycyl-DL-alanine (0·28 g.), ammonium thiocyanate (0·14 g.), and acetic anhydride containing 10% of acetic acid (1 c.c.) were heated at 100° for 20 minutes. The *thiohydantoin* (0·235 g.; m. p. 192°) was collected and washed with acetic anhydride, then ether–light petroleum. The m. p. was not raised by recrystallisation from aqueous ethanol (Found: C, 52·2; H, 4·8; N, 12·6; S, 10·0. $C_{14}H_{14}O_4N_2S$ requires C, 52·3; H, 4·7; N, 13·1; S, 10·0%). The thiohydantoin is sparingly soluble in water and soluble in most organic solvents; it is sparingly soluble in acetic acid–sodium acetate buffer (pH 5), and can thus be differentiated from the petide (I) which readily dissolves in the buffer.

(2) Ammonium thiocyanate (0·25 g.) and acetic anhydride (11·25 c.c.) were added to carbobenzyloxyglycylalanine (0·25 g.) in warm formic acid (1·25 c.c.). After 48 hours the reaction mixture was diluted with water, and the thiohydantoin collected (yield, 0·13 g.; m. p. 192°).

(3) Ammonium thiocyanate (0·05 g.) and acetic anhydride containing 10% of acetic acid (0·3 c.c.) were added to carbobenzyloxyglycylanine (0·144 g.) in formamide (0·38 c.c.). After being kept overnight, the thiohydantoin was isolated from the diluted reaction mixture and recrystallised from 75% ethanol; the yield was 0·04 g., and the m. p. 188—191°.

(4) Ammonium thiocyanate (0·066 g.) and acetic anhydride containing 33% of dichloroacetic acid (0·7 c.c.) were added to carbobenzyloxyglycylalanine (0·13 g.). After 13 minutes at 100°, ice and 2 c.c. of 50% sodium acetate solution were added; the crude product (0·123 g.; m. p. 164—168°) gave 0·039 g. of thiohydantoin after recrystallisation. If heating were carried out for only 4 minutes, the crude product (0·1 g.; m. p. 178—182°) was less impure. Similar results were obtained after 1½ hours at 50°.

(5) Carbobenzyloxyglycylalanine (0·219 g.) and ammonium thiocyanate (0·12 g.) were heated in acetic anhydride containing 20% of dichloroacetic acid at 100° for 10 minutes. The thiohydantoin (m. p. 192°) separated in a yield of 0·156 g.

(6) Carbobenzyloxyglycylalanine (0·208 g.) was heated with benzoyl *iso*thiocyanate (0·5 c.c.) at 100° for 1 hour and then at 120° for 2 hours. The reaction mixture was diluted with benzene. The product, recrystallised twice from 75% ethanol, had m. p. and mixed m. p. with an authentic sample of thiohydantoin, 192°.

Conversion of 5-Methyl-2-thiohydantoin into Alanine.—5-Methyl-2-thiohydantoin (4·9 mg.) was heated with constant-boiling hydrobromic acid (0·1 c.c.) at 150° for 6 hours. After dilution with water to 2 c.c., 0·005 c.c. was transferred to Whatman number 54 filter paper. The same volumes of several solutions of alanine of known concentration were also put on the same paper, and after equilibration overnight the chromatogram was developed with phenol–water (4 : 1). The yield of alanine was estimated by Boissonas's method (*loc. cit.*) to be 90%. In a similar experiment in which the time of heating was extended to 22 hours the yield was 88%.

Conversion of 1-Carbobenzyloxyglycyl-5-methyl-2-thiohydantoin into Alanine.—The thiohydantoin (7 mg.) in 0·1N-sodium hydroxide (0·7 c.c.) was kept for 20 minutes at 0°, then brought to pH 8 by passing in carbon dioxide and extracted thrice with ethyl acetate. After drying, the solvent was distlled, and the residue heated with constant-boiling hydrobromic acid (0·2 c.c.) overnight at 155°. The amount

* [*Added in proof.*] Chibnall and Rees (*Biochem. J.*, 1951, **48**, xlvii) and Fromageot, Jutisz, Meyer, and Pénasse (*Compt. rend.*, 1950, **230**, 1905) have also shown alanine to be a terminal amino-acid in insulin.

of alanine in the hydrolysate (which was free from glycine) was estimated, as described above, to be 82%. In a second experiment, the yield was 86%.

After the extraction with ethyl acetate, the aqueous layer was acidified to pH 2 and again extracted with ethyl acetate. When treated as above, glycine, but no alanine, was detected in the hydrolysate.

The Action of Acetic Anhydride and Ammonium Thiocyanate on Insulin.—(1) Insulin was acetylated by cautious addition of acetic anhydride to the protein dissolved in aqueous potassium hydrogen carbonate; the acetylated material separated from the acid solution. The dried product (18 mg.) was heated at 100° for 30 minutes with ammonium thiocyanate (3·5 mg.) and acetic anhydride (0·5 c.c.). The acetic anhydride was then distilled off *in vacuo*, and the last traces were removed by dissolving the residue in water, neutralising with ammonia, and drying in a vacuum-desiccator over phosphoric oxide and sodium hydroxide. The residue was treated with 0·1N-barium hydroxide (0·8 c.c.) for 15 minutes, and the solution neutralised with carbon dioxide and extracted with ethyl acetate. The solvent was distilled from the dried extract, and the residue hydrolysed with constant-boiling hydrobromic acid for 3 hours at 155°. The diluted hydrolysate was filtered and evaporated; analysis by chromatography on Whatman No. 4 paper, phenol–water being used as solvent, revealed the presence of alanine and possibly a small amount of aspartic acid. Comparison of the size of the alanine spot with spots from solutions of known concentrations indicated a yield of approximately 20 μg. of alanine.

Recovery of 5-Methyl-2-thiohydantoin in Presence of Insulin.—5-Methyl-2-thiohydantoin (0·19 mg.) in 0·1N-sodium hydroxide (0·5 c.c.) was added to acetylated insulin (12·6 mg.) in 0·1N-sodium hydroxide (0·5 c.c.). After 30 minutes the mixture was neutralised with carbon dioxide and extracted with ethyl acetate. The yield of alanine after hydrolysis with hydrobromic acid was 17% based on the amount of 5-methyl-2-thiohydantoin present.

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531. *Hydrated Calcium Silicates. Part II.* Hydrothermal Reactions: Lime : Silica Ratio 1 : 1.*

By L. HELLER and H. F. W. TAYLOR.

Calcium silicate hydrate (I), $CaO,SiO_2,aq.$–$3CaO,2SiO_2,aq.$ (*J.*, 1950, 3682), has now been prepared hydrothermally in a crystalline condition; its *X*-ray powder pattern shows many lines which are not disclosed by samples prepared at room temperature and can be assigned indices on the basis of the unit cell already tentatively proposed; but single-crystal photographs will probably be necessary to determine the unit cell with certainty.

At 110°, calcium silicate hydrate (I) appears to be the final product of hydrothermal treatment of mixtures of lime : silica ratio 1 : 1. At higher temperatures, up to at least 320°, it is obtained only as an intermediate product, more prolonged treatment yielding other species. At 135—180°, the compound CaO,SiO_2,H_2O, described by Flint and his co-workers, is obtained as a final product and is distinct from calcium silicate hydrate (I). At 160—300°, xonotlite is produced. The evidence that xonotlite contains zeolitic water is shown to be inconclusive. At 350° a product was obtained which gave the *X*-ray powder pattern of xonotlite together with certain additional lines, indicating either a new modification of xonotlite or the presence of a second as yet unidentified phase.

IN Part I of this series * it was shown that calcium silicate hydrate (I) could be formed in the CaO–SiO_2–H_2O system at room temperature, and that its composition varied between approximately $CaO,SiO_2,aq.$, and $3CaO,2SiO_2,aq$. Although the *X*-ray powder data gave some indications of the structure, further investigation was not possible because of the very small size of the crystals and partial disorder in their structure.

Foret (*Compt. rend.*, 1936, **203**, 80; 1937, **204**, 977) showed that, on hydrothermal treatment at 100—140°, mixtures of lime : silica ratio 1 : 1 yielded a product having an *X*-ray pattern similar to that of material prepared at room temperature by Chassevent (*ibid.*, 1934, **199**, 673). As was shown in Part I, the latter material probably consisted of calcium silicate hydrate (I) mixed with calcite. Inspection of Foret's data suggests the same conclusion, only three of the spacings quoted (3·0, 2·8 and 1·85 A.) being unambiguously attributable to calcium silicate hydrate (I). Nevertheless, the results suggest that this compound can be formed under

* Part I, *J.*, 1950, 3682.

hydrothermal conditions and it appeared possible that a more detailed investigation might enable it to be prepared in a more crystalline condition and in a relatively pure state.

At higher temperatures, up to approximately 350°, all previous investigators have found the hydrothermal treatment of mixtures of lime : silica ratio 1 : 1 to yield xonotlite ($CaO,SiO_2,0{\cdot}2$ or $0{\cdot}25H_2O$), whereas wollastonite is produced at 400° or above; for references and discussion see Taylor and Bessey (*Mag. Concrete Res.*, 1950, **1**, No. 4, 15). Flint, McMurdie, and Wells (*J. Res. Nat. Bur. Stand.*, 1938, **21**, 617) obtained at 150° a further compound which they believed to be CaO,SiO_2,H_2O. The *X*-ray data for this compound (McMurdie and Flint, *ibid.*, 1943, **31**, 225) show a certain resemblance to those of calcium silicate hydrate (I), but it is not clear from their data whether a well-crystallised form of the latter compound or a further distinct species had been produced.

The present investigation was carried out (i) to determine whether the crystallisation of calcium silicate hydrate (I) could be improved so as to obtain material suitable for a more detailed *X*-ray investigation, and (ii) to establish the relation of this compound to these obtained at higher temperatures, and especially with the CaO,SiO_2,H_2O reported by Flint, McMurdie, and Wells.

Experimental.

Methods.—Hydrothermal reactions were carried out in stainless steel pressure vessels in an electric oven at 110—350°. The quantity of water was always such that both liquid and vapour phases were present. At temperatures above 100° the solubilities of both lime and silica are small (Flint, McMurdie, and Wells, *loc. cit.*), and the pressure developed is therefore practically equal to the saturated vapour pressure of steam at the operating temperature. For the same reason, the lime : silica ratio of the solid product is almost that of the starting materials. These were in some cases mixtures of calcium hydroxide and silica gel, and in others calcium silicate hydrate (I) which had been prepared by one of the methods described in Part I. In all cases the lime : silica molar ratio of the starting material was approx. 1 : 1, and the solid and liquid phases were in contact throughout the reaction.

After the hydrothermal treatment, the reaction mixture was filtered. The solid residue was washed with a minimum of water followed by acetone, and dried in a vacuum-desiccator over anhydrous calcium chloride. The solid was investigated optically and by *X*-ray powder photographs.

Starting Materials.—The calcium silicate hydrate (I) samples were prepared by methods already described (Part I, *loc. cit.*). The silica gel was the sample described in the same paper. A total dry weight of 150—200 mg. was used in each experiment, together with 5 ml. of water.

Hydrothermal Reactions.—The reaction mixtures were placed in silver test-tubes of height 3" and external diameter ½". These fitted into stainless steel tubes based on a design by Barrer (*J.*, 1948, 127). The tubes were made from hexagonal bar of width 1" across the flat, and length 5½". An axial hole 4" deep and 17/32" in dameter was bored into one end. The first ¾" of the hole was tapped with a ½" Whitworth thread, and a screw cap made to fit from the same material as the tube. An annealed copper washer ¼" thick formed the gasket. Knife-edged ridges of approx. 0·015" in height were turned on the surfaces of the tube and the cap; these bit into the copper washer.

The tubes were heated in an electric oven whose temperature could be regulated to within ±2°.

While the general trend of the results, as shown in Table I (below), was clear, the rate of the reactions was to some extent irreproducible. Apparently identical samples, which received identical treatment, reacted to different extents; some mixtures failed to crystallise appreciably. These have been excluded from Table I. It was found that badly crystallised samples, possibly on account of their higher pH values, showed a greater tendency to absorb atmospheric carbon dioxide during the isolation of the solid product.

X-Ray Apparatus.—A sealed, filament *X*-ray tube with copper target was operated at approx. 35 kv. and 15—20 mA. Photographs were taken with a powder camera of 19-cm. diameter, with a nickel-foil filter in contact with the film to remove $K\beta$ and fluorescent radiations. Exposures of about 20 hours were normally required, Ilfex double coated film being used; with the recently available Ilford Industrial G film this was reduced to about 8 hours. In a few cases, in order to observe the longer spacings clearly, single-crystal cameras of 6-cm. diameter were used. Spacings were determined by use of a travelling microscope, and are probably correct to within ±0·01 A. at 1·5 A. or below, rising to ±0·02 at 3 A. and ±0·1 A. at 6 A. Intensities were estimated visually.

Results and Discussion.

The products obtained from each experiment, together with the experimental conditions, are given in Table I. Xonotlite, where present, was identified by its optical properties and *X*-ray powder data (Taylor and Bessey, *loc. cit.*), as were calcium carbonate and hydroxide. The characterisation of the remaining species is discussed later.

Calcium Silicate Hydrate (I).—Several of the experiments (nos. 88, 82, 47, and 62) yielded *X*-ray photographs which, apart from the presence of calcium carbonate and/or hydroxide lines in some cases, were identical with that of calcium silicate hydrate (I) prepared at room temperature (Taylor, *loc. cit.*). This compound can therefore be produced under hydrothermal conditions. At 110° it was present even after prolonged treatment, but as explained later it

TABLE I.

Results of hydrothermal experiments.

Expt. no.	Temp. of prepn.	Starting material.*	Time of prepn. (days).	Products.§
15	350°	P	7 †	Xonotlite + A
88	320	M	½	CSH(I) + CC
13	300	P	4	Xonotlite
14	300	P	4	Xonotlite
6	240	P	6	Xonotlite
36	200	P	5	CSH(I) ‖
46	200	P	60 ‡	Xonotlite
11	200	P	30	Xonotlite
12	200	M	30	Xonotlite
82	180	M	2	CSH(I) + CC
91	180	M	42	CSH(I) + B
93	180	M	28	CSH(I) + B + xonotlite
92	180	M	14	Xonotlite
18	160	P	16	CSH(I) ‖
21	160	P	30	Xonotlite + B
23	160	P	36	Xonotlite + B
47	150	M	35	CSH(I) + CH + CC
62	140	M	60	CSH(I) + CH + CC
67	140	Q	27	CSH(I) ‖
80	140	M	75	CSH(I) ‖ + B
78	140	Q	75	CSH(I) ‖ + B
51	135	M	60	CSH(I) + B
39	110	M	63	CSH(I) ‖
58	110	M	200	CSH(I) ‖

* M = mixture of $Ca(OH)_2$ and SiO_2 gel; P or Q = calcium silicate hydrate (I) made by double decomposition or from silica gel respectively (Taylor, *loc. cit.*). † Preceded by 28 days at 200° and then 6 days at 300°. ‡ Preceded by 35 days at 150°. § CSH(I) = calcium silicate hydrate (I); CH = calcium hydroxide; CC = calcium carbonate; A, B, see text. ‖ In a well-crystallised form; see text.

was in this case in a better state of crystallisation, giving a more complete *X*-ray powder diagram. At higher temperatures it was always observed in the products obtained after shorter periods (Expts. 82, 36, and 88, at 180°, 200°, and 320° respectively), whereas more prolonged treatment yielded other species, notably xonotlite. This was so whether a mixture of calcium hydroxide and silica gel, or calcium silicate hydrate (I) made at room temperature, was used as the starting material. At temperatures above about 130°, calcium silicate hydrate (I) is therefore an intermediate product in the formation of other species.

In some cases (Expts. 67, 36, 39, and 58) many additional *X*-ray lines were observed and the pattern as a whole was much sharper. The data for a typical sample are given in Table II. With one minor exception, these lines always occurred with the same intensities relative both to each other and to the original lines. This was also largely true for preparations of higher lime : silica ratio in which the same pattern was obtained (Heller and Taylor, unpublished work). It therefore appeared probable that the additional lines represented spacings which, on account of imperfect crystallisation, did not occur in the first set of preparations, or in those made at room temperature. This was supported by the results of optical examinations. The preparations showing only the original lines appeared amorphous, but those showing the full pattern consisted of minute irregular crystals which were weakly birefringent and had mean refractive indices of 1·55—1·565. The refractive index appeared to vary slightly with both the temperature of preparation and the degree of crystallinity, the more crystalline preparations and those made at higher temperatures tending to have the higher values. It is possible that the refractive index of the sample may depend on its water content. The largest crystal observed (in sample 36) was 30 × 10 × 10 μ., but the crystals were usually very much smaller (about 3 μ.), and thus not suitable for more complete optical investigation.

Table II also includes spacings and intensities calculated on the basis of the unit cell already tentatively suggested (Taylor, *loc. cit.*). While many of the observed lines can be accounted for, the agreement is less satisfactory in other cases, and it is possible that the true cell may be larger or less symmetrical than that proposed. More conclusive results than this can scarcely be obtained in the absence of single-crystal photographs. No attempt has been made to index spacings shorter than 2·0 A., except for the *hk*0 lines, as the possibility of finding a fit increases rapidly with diminishing spacing, so that any comparison beyond this point would be of little significance.

TABLE II.

X-Ray data for calcium silicate hydrate (I).

Obsd. (sample 58).		Calc.*		Obsd. (sample 58).	
Spacing, A.	Intensity.	Spacing, A.	Indices.	Spacing, A.	Intensity.
11·0	s †	11·0	001	1·56	vvw
5·5	ms †	5·5	002	1·55	mw †
3·49	vvw	3·50	011	1·52	vw
3·29	vvw	—	?	1·50	w
3·07	vs †	3·07	110	1·432	mw
2·97	vs	2·95	111	1·406	mw †
2·81	vs †	2·81	200	1·398	vvw
2·52	w	2·49	202	1·369	vvw
2·48	w	2·46	104	1·312	vvw
2·41	vw	2·35	113	1·295	vvw
2·28	ms ‡	2·23	203	1·281	vvw
2·22	vw	2·20	005, 014	1·259	vvw
2·14	ms	2·17	211	1·245	w †
2·07	ms	2·06	212	1·214	vw
2·00	ms	2·04	105, 114	1·188	vw †
1·88	vw	—	—	1·163	vw
1·83	vs †	1·83	020	1·138	mw
1·80	w	—	—	1·112	w †
1·76	vw	—	—	1·073	mw †
1·73	vw	—	—	1·058	vvw
1·67	vs †	1·67	310	1·043	vvw
1·62	w	—	—	1·024	vw
1·60	vw				

* Spacings calculated on the basis of an orthorhombic cell having $a = 5{\cdot}62$ A., $b = 3{\cdot}66$ A., $c = 11{\cdot}0$ A. The spacing or spacings nearest to the observed value are given.

† Lines shown also by the imperfectly crystallised samples. The spacings given here for these lines differ slightly from those recorded previously; this is the result of more accurate measurement and does not represent any real difference.

‡ This line varied in intensity for different samples, being sometimes completely absent.

Xonotlite.—The present results show that xonotlite is formed, either from calcium silicate hydrate (I) or from lime–silica mixtures, over the range 160—350°. Between 200° and 300° it appears to be the sole final product, but outside this range it may be mixed with other species. This is compatible with the previous investigations.

The *X*-ray results were in close agreement with those recorded by earlier workers (Taylor and Bessey, *loc. cit.*). Whenever xonotlite was obtained, whether pure or mixed with another species, no variations could be detected in either the spacings or the relative intensities of any of its lines. A sample of the natural mineral also gave a photograph indistinguishable from those of the synthetic samples. We are thus unable to confirm the view expressed by Clark and Bunn (*J. Soc. Chem. Ind.*, 1940, **59**, 155) and by Reinhard, Brandenberger, and Oesterheld (*Helv. Chim. Acta*, 1942, **25**, 336) that the xonotlite pattern shows significant differences between natural and synthetic samples.

There has been no general agreement regarding the water content of xonotlite. Most of the early workers used the formula $5CaO,5SiO_2,H_2O$ but Nagai (*Z. anorg. Chem.*, 1932, **206**, 177; **207**, 321) and Jander and Franke (*ibid.*, 1941, **247**, 161) considered it to be $4CaO,4SiO_2,H_2O$. More recently it has been suggested that the water content is variable (Reinhard, Brandenberger, and Oesterheld, *loc. cit.*), and Alcock, Clark, and Thurston (*J. Soc. Chem. Ind.*, 1944, **63**, 292) describe the compound as zeolitic. The former workers based their view on changes in the *X*-ray spacings, which, as stated above, we have been unable to confirm. We have also failed to note a shortening of the xonotlite spacings, as described by Reinhard *et al.*, on heating to 280° for 3 days a preparation made at 160°. Apart from this the view seems to have been suggested by the observation of Foret (*loc. cit.*) that the water contents of synthetic samples dried at 100° decrease as the temperature of preparation is raised. The present results suggest, however, that the products obtained by Foret at 140° or slightly higher temperatures may have contained calcium silicate hydrate (I), which almost certainly has a higher water content than has xonotlite. Owing to imperfect crystallisation, this compound may be present to an appreciable extent without being apparent in the *X*-ray pattern. Thus, while the available evidence does not rule out the possibility of a variable water content, there seems to be no valid reason for postulating this. Most of the previous determination of the water content are in any case of uncertain value owing to the use of inadequately defined conditions of drying. An investigation under isobaric conditions is in progress.

X-Ray Lines " A ".—The preparation obtained at 350° showed in its *X*-ray pattern, besides xonotlite, a number of additional lines which are listed in Table III. They do not coincide with the spacings of either α-(pseudo-) or β-(monoclinic) wollastonite (A.S.T.M. index, and Ehrenberg, *Z. physikal. Chem.*, **1931**, *B*, **14**, 425, respectively; confirmed by us). Two explanations appear possible: a second species together with xonotlite, or a slightly modified crystalline form of xonotlite, may have been produced. Optical examination showed that most of the sample consisted of very fine needles, having the usual optical properties of xonotlite. A few crystals of higher refractive index could be detected but it is doubtful if they were present in sufficient quantity to account for the extra lines " A." Thus it is not possible to choose between the alternative interpretations on the basis of the available evidence.

X-Ray Lines " B ".—Experiments 21 and 23 at 160° gave the *X*-ray pattern characteristic of xonotlite together with a number of extra lines. These are listed in Table IV. Other preparations (51, 78, 80, and 91) showed the lines of calcium silicate hydrate (I) with the same extra lines, whilst preparation 93 showed these together with both xonotlite and calcium silicate hydrate (I). The extra lines were in all cases of the same intensities relative to each other but not to those of the xonotlite or calcium silicate hydrate (I). These facts, and particularly the occurrence of the lines " B " in conjunction with those of either or both of two other species, strongly suggested the presence of a further compound. This was confirmed optically. The above preparations all contained thin needles of mean refractive index 1·60 and negative elongation, in addition to crystals of xonotlite and/or calcium silicate hydrate (I) in accordance with the *X*-ray data.

TABLE III.

X-Ray data; lines " A."

Spacing, A.	Intensity.	Spacing, A.	Intensity.	Spacing, A.	Intensity.
6·25	v.w.	3·49	m.	2·09	v.v.w.
4·71	m.	3·14	v.s.	1·92	v.v.w.
3·85	m.	3·00	v.v.w.	1·80	m.
3·76	v.w.	2·43	w.		

TABLE IV.

X-Ray powder data; lines " B ".

This investigation; lines " B ".		McMurdie and Flint; CaO,SiO_2,H_2O.		This investigation; lines " B ".		McMurdie and Flint; CaO,SiO_2,H_2O.	
Spacing, A.	Intensity.	Spacing, A.	Intensity.	Spacing, A.	Intensity.	Spacing, A.	Intensity.
6·04	m.w.	6·13	v.w.	2·10	v.v.w.	2·116	v.w.
4·51	v.v.w.	4·52	w.	2·01	m.w.	2·013	w.
4·23	v.v.s.	4·24	w.	1·925	v.w.	—	—
3·75	m.	3·80	v.w.	1·893	s.	1·893	s.
3·56	m.	3·58	w.	1·867	v.v.w.	1·868	v.w.
3·21	s.	3·21	m.	1·805	v.v.w.	1·808	v.w.
3·03	v.v.s.	3·01	v.s.	1·773	m.w.	1·772	m.
2·78	m.s.	2·78	m.	1·631	v.v.w.	1·632	v.w.
2·50	s.	2·50	m.	1·605	m.w.	1·601	m.
2·24	s.	2·23	s.				

Both the optical and the *X*-ray data (Table IV) for this species are in close agreement with those recorded by Flint and his co-workers (*loc. cit.*) for the compound CaO,SiO_2,H_2O. There are some discrepancies for the relative intensities, notably in the case of the 4·23-A. line, but the agreement is sufficiently close to establish that the compounds are identical. Our attempts to obtain the pure compound, unlike those by Flint, McMurdie, and Wells, were unsuccessful. The present results show, however, that this compound is quite distinct from calcium silicate hydrate (I) and that it is not a well-crystallised form of it.

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532. *Studies in the Sterol Group. Part LII.* Infra-red Absorption of Nuclear Tri- and Tetra-substituted Ethylenic Centres.*

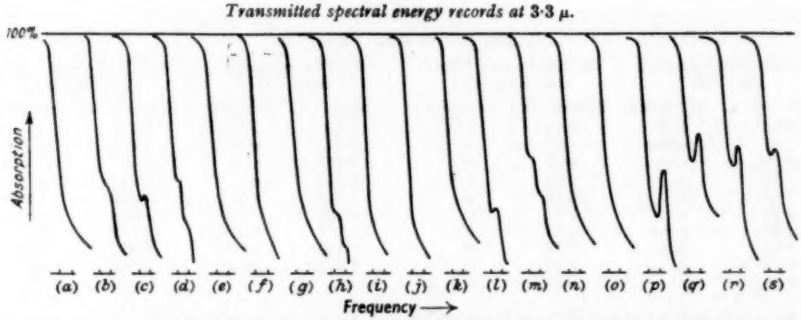
By Peter Bladon, Joyce M. Fabian, H. B. Henbest, (the late) H. P. Koch, and Geoffrey W. Wood.

The infra-red spectra of nineteen singly unsaturated steroids, providing examples of all the possible tri- and tetra-substituted ethylenic positions in the nucleus except Δ^{16}, have been recorded in the three characteristic olefinic-absorption regions of 3·3, 6, and 10—15 μ. under comparable conditions of path-length and concentration. The tetra-substituted ethylenes (hydrocarbons, alcohols, and ketones) are transparent at these wave-lengths, whereas the tri-substituted derivatives display more or less characteristic absorption bands of weak or moderate intensity in all three spectral regions. The occurrence of a tri-substituted ethylenic centre can therefore in general be recognized, and its location further specified, by means of significant frequency and intensity differentiation at 3·3 and at 6 μ. The electronic nature of steric strain accompanying the presence of unsaturation in rings C or D of the steroid nucleus, and its spectroscopic consequences, are discussed.

The location of unconjugated ethylenic double bonds in the nucleus of synthetic or naturally occurring compounds of the sterol group presents a diagnostic problem which clearly demands a thorough exploration of the possibilities inherent in spectroscopic techniques. So far, little systematic work has been done in this field. Ultra-violet studies have been restricted to the

Fig. 1.

Transmitted spectral energy records at 3·3 μ.



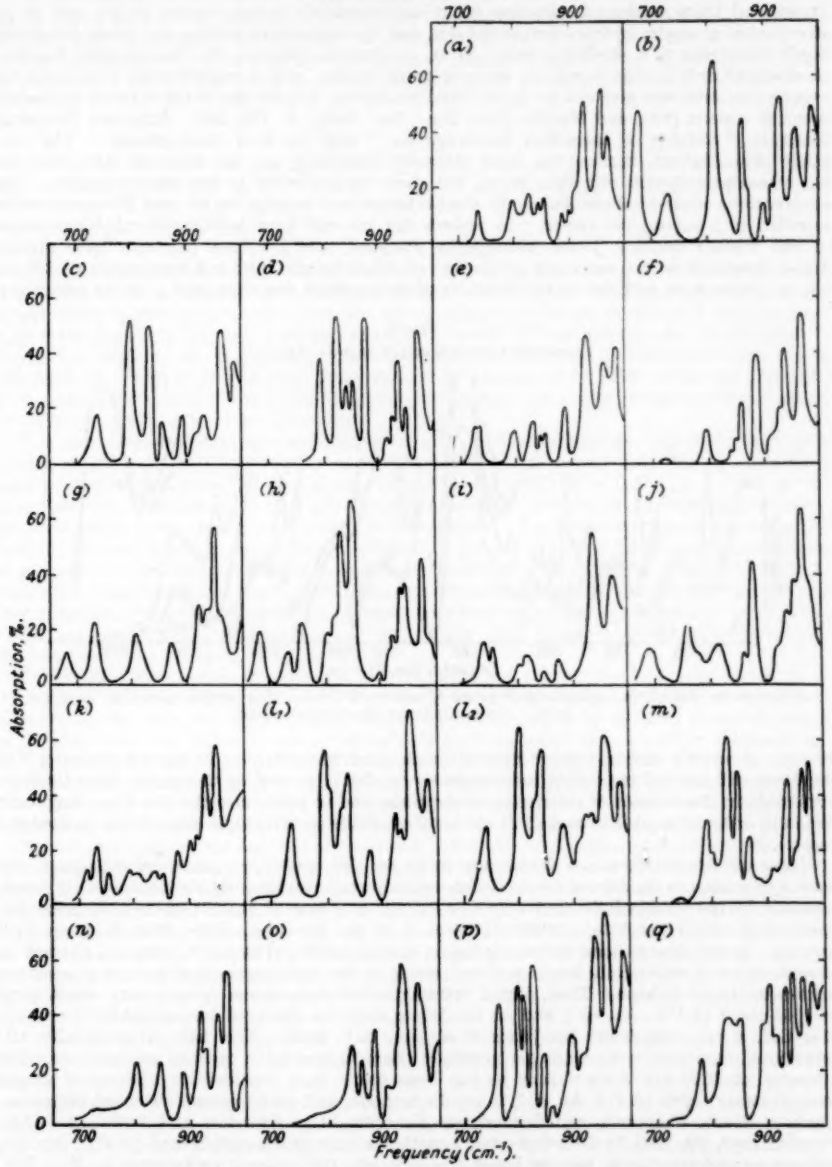
Calibration marks refer to 3030 *and* 3040 *cm.*$^{-1}$. *Solutions in carbon tetrachloride: effective path-length of solute* ~0·035 *mm. For sample references, a—s, see table.*

instrumentally more accessible absorption region of conjugated double bonds; quartz ultra-violet measurements on unconjugated ethylenic centres, undertaken here concurrently with the present work, will be reported in a separate communication. In the infra-red region, well-tried diagnostic methods were available only for non-cyclic compounds when this project was initiated, but a notable contribution to the characterization of isolated double bonds in steroid molecules was more recently published by Jones, Humphries, Packard, and Dobriner (*J. Amer. Chem. Soc.*, 1950, **72**, 86). These authors investigated the 6-μ. region of isolated Δ^{2}-, Δ^{5}-, Δ^{7}-, $\Delta^{5(10)}$-, $\Delta^{9(11)}$-, Δ^{11}-, Δ^{14}-, and Δ^{16}-ethylenic centres in a number of steroid molecules (mostly containing carbon–oxygen double bonds). They also reported some preliminary data on the 3·3-μ. region (cf. Jones, Williams, Whalen, and Dobriner, *ibid.*, 1948, **70**, 2024) and drew attention to the diagnostic possibilities of the 10—12-μ. range.

* Part LI, *J.*, 1951, 1190.

FIG. 2.

Absorption spectra at 10—15 μ.

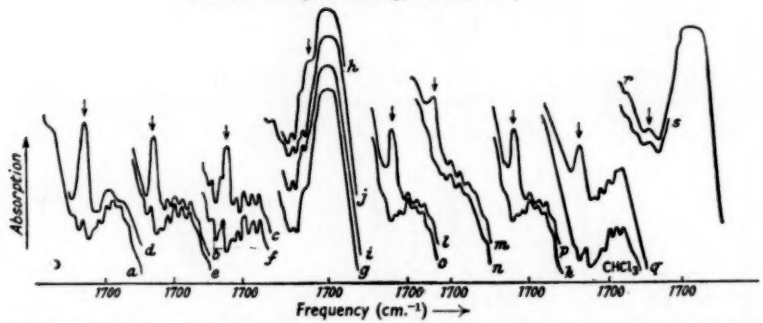


a—l_1, p, q in carbon disulphide solution : l_2, m—o in Nujol suspension. Effective path-length of steroid sample ~0·045 mm. For sample references, a—q, see table.

Characteristic infra-red absorption of olefinic groupings arises from the stretching vibrations of the double bonds and of any adjacent C–H bonds (near 6 and 3·3 μ. respectively), and also from the out-of-plane bending vibrations of these olefinic C–H bonds (at 10—15 μ.). As is well known, all three modes of vibration occur approximately independently of the rest of the structure in aliphatic hydrocarbon molecules, and this appears to be true also when the double bonds form part of a strainless ring. Thus, *cyclo*hexene displays the characteristic bands of *cis*-disubstituted double bonds in straight-chain olefins, and 1-methyl- and 1-methyl-4-*iso*propenyl-*cyclo*hexene give rise to typical trialkylethylene frequencies in the three characteristic spectral regions (Fox and Martin, *Proc. Roy. Soc.*, 1940, *A*, **175**, 208; American Petroleum Institute, "Catalog of Infra-Red Spectrograms;" and our own observations). The most easily characterized, because the most intensely absorbing, are the terminal methylene and the *trans*-disubstituted ethylenic types, but these do not occur in the steroid nucleus. Our observations on *cis*-disubstituted cyclic double bonds such as occur in Δ^{2}- and Δ^{11}-sterols will be reported in a subsequent paper. As regards the tri- and tetra-substituted ethylenic centres in the steroid nucleus, Jones, Humphries, Packard, and Dobriner (*loc. cit.*) have already drawn attention to the weakness or absence of characteristic infra-red frequencies at 3·3 and 6 μ. in many cases, and also to the difficulty of their certain detection at 6 μ. in the presence of

FIG. 3.

Transmitted spectral energy records at 6 μ.



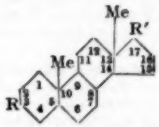
Solutions in chloroform: effective path-length of solute ~0·2 mm. For sample references, a—s, see table. Arrows indicate characteristic bands.

strongly absorbing carbon–oxygen double bonds or of water-vapour in the spectrometer.[7] It therefore still seemed important independently to check, as well as to extend, their findings, preferably in the absence of interfering absorptions, and to study also the 10—15-μ. diagnostic region in order to be able to make full use of all available spectroscopic information in doubtful cases.

Infra-red Spectra–Structure Correlation Rules for Tri- and Tetra-substituted Ethylenes, and their Application to the Steroid Group.—Tetra-substituted ethylenes do not contain C–H bonds adjacent to the double bond and therefore cannot give rise to characteristic absorption frequencies in either the 3·3-μ. (3000—3100-cm.$^{-1}$) or the 10—15-μ. (650—1000-cm.$^{-1}$) spectral regions. In the double-bond stretching region at 6-μ. (1620—1700-cm.$^{-1}$), infra-red absorption of such olefins is excessively weak or absent owing to the high degree of symmetry around the tetra-substituted linkage. Thus, liquid tetramethylethylene shows only a very small band at 1684 cm.$^{-1}$ (A.P.I., *loc. cit.*), and no maximum could be observed for cholest-8(14)-enol (I; R = OH, C_8–C_{14} ethylenic) by Jones *et al.* (*loc. cit.*, 1950). The less symmetrically trisubstituted ethylenic compounds are generally characterized by a band of moderate to weak intensity at 1660—1675 cm.$^{-1}$ such as has been found in a considerable number of simple straight-chain olefins (A.P.I., *loc. cit.*), in acyclic terpenes and polyisoprenes (Barnard, Bateman, Harding, Koch, Sheppard, and Sutherland, *J.*, 1950, 915; Saunders and Smith, *J. Appl. Physics*, 1949, **20**, 953), in 1-methyl- and 1-methyl-4-*iso*propenyl-*cyclo*hexene (A.P.I., *loc. cit.*, and our own observations), and by Jones *et al.* (*loc. cit.*, 1950) also in cholesterol (I; R = OH, C_5–C_6 ethylenic) and cholest-7-enol (I; R = OH, C_7–C_8 ethylenic). The single olefinic C–H group of trisubstituted ethylenes gives rise to moderate or weak characteristic absorption in

the 3·3-μ. C–H stretching region near 3050 cm.$^{-1}$ (Fox and Martin, *loc. cit.*; Saunders and Smith, *loc. cit.*; cf. also Jones *et al.*, *loc. cit.*, 1948), and also to a well-known moderate or strong and rather variable out-of-plane bending frequency at 790—850 cm.$^{-1}$ (12 μ.) which apparently lies at 796—798 cm.$^{-1}$ in 1-methyl- and 1-methyl-4-*iso*propenyl-*cyclo*hexene (A.P.I., *loc. cit.*, and our own observations), at 790 cm.$^{-1}$ in α-pinene (II) (A.P.I., *loc. cit.*), and at 803 or 844 cm.$^{-1}$ in cholesterol (I; R = OH, C_5–C_6 ethylenic) (Baird, O'Bryan, Ogden, and Lee, *J. Opt. Soc. America*, 1947, **37**, 754; Hainer, King, and McMahon, *Physical Rev.*, 1949, **75**, 1320).

(I.)



(II.)

Chemical methods can usually be applied to establish the presence or otherwise of a double bond in the sterol nucleus without much difficulty. Ideally, then, we might expect the tetra-substituted ethylenic groups to be uniquely characterized by relative or complete transparency in all three diagnostic frequency regions and the $\Delta^{8(9)}$- and $\Delta^{8(14)}$-compounds to be indistinguishable in this respect; we might expect the trisubstituted ethylenic derivatives to give bands near 3050 cm.$^{-1}$ and to be uniquely characterized by absorption at 1660—1675 and 790—850 cm.$^{-1}$ with possibly minor characteristic variations amongst the several members of this group (Δ^{4}-, Δ^{5}-, Δ^{7}-, $\Delta^{9(11)}$-, Δ^{14}-, or Δ^{16}-ethylenic).

The above spectra–structure correlation rules for olefinic groupings appear to have been strictly established for hydrocarbons only, and it therefore seemed desirable to examine a number of pure samples of steroid hydrocarbons (I; R = H, R′ = C_nH_{2n+1}) as well as the usual functional derivatives, so that any disturbing influence of functional groups on the olefinic spectrum might be recognized and, if need be, eliminated. Exploratory infra-red spectroscopic work in the sterol group is also greatly facilitated by the use of purely hydrocarbon compounds on account of their ready solubility in spectroscopically suitable solvents. It is preferable to work with solutions rather than solid films or suspensions in order that relative absorption intensities may be more conveniently estimated and compared between different substances, crystal structure effects eliminated, and sufficiently long effective path-lengths employed without undue loss of radiation by scattering.

Preparative Work.—Steroid hydrocarbons containing a double bond at positions 4, 5, 7, 8(9), and 8(14) have been prepared. Since hydroxyl groups appear to exert no detectable influence on the infra-red absorption of double bonds separated by at least 3 carbon atoms (cf. ergost-7-ene and 3β-hydroxyergost-7-ene), 3β-hydroxyergost-14-ene and 24-hydroxychol-9(11)-ene were prepared as examples of their respective double-bond types.

The route employed for preparing the parent hydrocarbons containing double bonds in the 7-, 8(9)-, and 8(14)-positions was to oxidise the readily accessible 3β-sterols to the corresponding ketones, which were reduced to hydrocarbons by the Kishner–Wolff method.

The chosen starting material for ergost-7- and 8(14)-ene was α-dihydroergosterol, this compound being obtained as a by-product from the irradiation of ergosterol which normally contains a few units % of the α-dihydro-compound as an impurity unaffected by the irradiation process. Hydrogenation of the side-chain double bond in α-dihydroergosterol to give 3-hydroxyergost-7-ene was effected in excellent yield in the presence of a Raney nickel catalyst at normal temperature and pressure, this method being found superior in yield and reproducibility to those employing platinum oxide catalysts. Use of a Raney nickel catalyst at higher temperatures and pressures resulted in isomerization of the nuclear double bond to the 8(14)-position, good yields of 3-hydroxyergost-8(14)-ene being obtained.

Oxidation of the above sterols to the corresponding ketones was carried out by chromic acid in acetone (cf. Bowden, Heilbron, Jones, and Weedon, *J.*, 1946, 39). No attack on the double bonds was then detected, the ketones being formed in good yield, accompanied by some unchanged sterol. The Kishner–Wolff reductions proceeded smoothly. The properties of ergost-8(14)-ene agreed with those given for this compound by Heilbron, Spring, and Webster (*J.*, 1932, 1705), who prepared it by sodium–alcohol reduction of the 3-chloro-compound.

A hydrocarbon containing an 8(9)-double bond [cholest-8(9)-ene] was conveniently prepared from zymosterol, which was isolated from the non-saponifiable part of yeast fat *via* its 24 : 25-dibromide (cf. Heath-Brown, Heilbron, and Jones, *J.*, 1940, 1182). It was possible to increase

considerably the scale of the dibromide formation by suitable cooling during the addition of bromine. Hydrogenation of zymosterol under mild conditions gave 3β-hydroxycholest-8(9)-ene, oxidized in this instance by the Oppenauer method to the corresponding ketone. Kishner–Wolff reduction then gave cholest-8(9)-ene.

Experimental.

M. p.s were determined on a Kofler block and are corrected. Optical rotations were determined in chloroform solution in a semi-micro-tube of length 1 dm. at room temperature (18—25°) unless stated otherwise. Samples for analysis and determination of physical constants were dried *in vacuo* at 115° or at 20° below the m. p. The alumina (P. Spence, Grade O) used for chromatography had an activity between 2 and 3 on the Brockmann–Schodder scale (*Ber.*, 1941, **74**, 75).

Cholest-4-ene.—This was prepared by Raney nickel desulphurization of the dibenzyl mercaptal of 3-ketocholest-4-ene (method of Hauptmann, *J. Amer. Chem. Soc.*, 1947, **69**, 562). By use of the more active W4 grade of Raney nickel (Adkins and Pavlic, *ibid.*, 1946, **68**, 1471) desulphurization could be effected at 20°. The mercaptal (1 g.) in dioxan (50 c.c.) was stirred for 7½ hours at 20° with W4 Raney nickel (5 g.). The product after recrystallization had m. p. 79·5—81°, $[\alpha]_D$ +68° (*c*, 1·28) (lit., m. p. 78—79°, $[\alpha]_D$ +65°). Dr. D. H. R. Barton kindly informed us that such cholest-4-ene contained an impurity (probably cholest-3-ene), and therefore for further purification the above product was converted into its dibromide (Mauthner, *Monatsh.*, 1907, **28**, 1113), which after recrystallization from benzene–ethanol formed needles, m. p. 117—119° (Mauthner gives m. p. 116—117°). A solution of the dibromide (275 mg.) and sodium iodide (500 mg.) in acetone (10 c.c.) was heated under reflux for 30 minutes. Cooling and dilution with water gave a crystalline product, which after recrystallization from ethanol gave pure cholest-4-ene as needles, m. p. 82—83·5°, $[\alpha]_D$ +76° (*c*, 0·7).

Cholest-5-ene.—This was prepared by the method of Mauthner and Suida (*ibid.*, 1894, **15**, 85). The product obtained was purified by chromatography to give cholest-5-ene of m. p. 92·5—94°, $[\alpha]_D$ −56° (*c*, 1·01) (lit., m. p. 88—90°, $[\alpha]_D$ −56°).

3β-Hydroxycholesta-8(9) : 24-diene (Zymosterol).—This sterol was obtained *via* its dibromide from the non-saponifiable material from yeast fat after the bulk of the ergosterol had been removed by crystallization. Subsequent purification of the crude dibromide was facilitated by partial purification of this crude gummy mixture (1 kg.) by trituration with (*a*) ethanol (1·1 l.) and (*b*) methanol (750 c.c.). This gave a light brown solid (720 g.) containing 20—25% of ergosterol, estimated spectroscopically. It was found possible to increase considerably the scale of the next stage by suitable cooling. The purified sterol mixture (75 g.) in dry ($CaCl_2$) ether (1·5 l.) was cooled to −10°; a 10% solution of bromine in acetic acid (450 c.c.) was then added with vigorous shaking during 1 minute, the internal temperature being kept at −5° to −10° by external cooling with solid carbon dioxide. The mixture was then set aside in an ice-bath until the supernatant liquid became dark (5—10 minutes). The product was rapidly filtered off and washed with ethanol (30 c.c.) and then ether (100 c.c.). Recrystallization from ethanol–chloroform (3 : 1) and ethyl acetate gave zymosterol dibromide (7—8 g.) as needles, m. p. 157—158°, $[\alpha]_D$ +7° (*c*, 1·89). Heath-Brown, Heilbron, and Jones give m. p. 157°, $[\alpha]_D$ +7·4° (*c*, 3·3).

Debromination of the dibromide with zinc dust and absolute ethanol gave zymosterol, m. p. 109°, $[\alpha]_D$ +49° (*c*, 3·10); Heath-Brown *et al.* give m. p. 107—109°, $[\alpha]_D$ +49° (*c*, 1·4).

3β-Hydroxycholest-8(9)-ene.—A solution of zymosterol (3 g.) in benzene (150 c.c.) was shaken with hydrogen at atmospheric pressure in the presence of Raney nickel (3 g.) until no more was absorbed. Evaporation of the solvent, after removal of the catalyst by filtration through a sintered-glass funnel, gave a solid, which after recrystallization from methanol afforded the sterol (2·7 g.) as flat plates, m. p. 128°, $[\alpha]_D$ +49° (*c*, 2·79). Wieland, Rath, and Benend (*Annalen*, 1941, **548**, 19) give m. p. 129°, $[\alpha]_D$ +50°.

3-Ketocholest-8(9)-ene.—A solution of the foregoing sterol (2·2 g.) in dry acetone (20 c.c.) was added to aluminium *tert.*-butoxide (2·5 g.) in dry benzene (50 c.c.), the mixture then being heated under reflux for 8 hours. After the addition of water, the steroid was isolated with ether. Traces of mesityl oxide were removed by the addition of xylene (7·5 c.c.), followed by evaporation *in vacuo*. The product was chromatographed on alumina (100 g.). Development with benzene gave a product which after recrystallization from methanol yielded *3-ketocholest-8(9)-ene* (1·35 g.) as flat needles, m. p. 119·5—120·5°, $[\alpha]_D$ + 69° (*c*, 2·28) (Found: C, 84·0; H, 11·5. $C_{27}H_{44}O$ requires C, 84·3; H, 11·5%). Elution of the chromatogram with ether–methanol gave unchanged sterol (0·22 g.), m. p. 127°.

Cholest-8(9)-ene.—A mixture of 3-ketocholest-8(9)-ene (750 mg.), hydrazine hydrate (7 c.c. of 60% w/v), and sodium (1·25 g.) dissolved in ethanol (25 c.c.) was heated (autoclave) for 8 hours at 210—220°. The steroid was isolated with light petroleum (b. p. 40—50°), the solution being passed through a column of alumina (75 g.), which was developed further with light petroleum (b. p. 40—50°). Evaporation of the eluant gave a solid, which after recrystallization from methanol afforded *cholest-8(9)-ene* (420 mg.), m. p. 92—93·5°, $[\alpha]_D$ +56° (*c*, 1·09) (Found: C, 87·6; H, 12·55. $C_{27}H_{46}$ requires C, 87·5; H, 12·5%).

3β-Hydroxyergosta-7 : 22-diene (α-Dihydroergosterol).—Crude α-dihydroergosterol, obtained as a residue from the irradiation of ergosterol, was purified *via* its acetate. Recrystallization of the acetate from benzene–acetone gave platelets, m. p. 180—181°, $[\alpha]_D$ −23° (*c*, 2·0). Barton and Cox (*J.*, 1948, 1354) give m. p. 181°, $[\alpha]_D$ −20° (*c*, 1·78). Alkaline hydrolysis of the acetate gave α-dihydroergosterol (microscopic needles from chloroform–methanol), m. p. 176—178°, $[\alpha]_D$ −23° (*c*, 2·1). Barton and Cox give m. p. 176°, $[\alpha]_D$ −19° (*c*, 1·8).

3β-Hydroxyergost-8(14)-ene (α-Ergostenol).—The acetate of this sterol was prepared by hydrogenation of 3β-acetoxyergosta-7 : 22-diene, either in acetic acid–ether with a platinum oxide catalyst at 100°/100

atm., or by the following new method. The diene-acetate (2 g.) in ethyl acetate (300 c.c.) was stirred in an autoclave with hydrogen at 100°/100 atm. in the presence of Raney nickel (3·5 g.) for 12 hours. The catalyst was removed by filtration through a pad of alumina. Recrystallization from ethyl acetate–methanol yielded the pure acetate (1·8 g.) as plates, m. p. 110—112°, $[\alpha]_D$ −1° (*c*, 2·15). Barton and Cox (*J.*, 1948, 783) give m. p. 109°, $[\alpha]_D$ +1° (*c*, 2·13).

Alkaline hydrolysis of the acetate gave 3β-hydroxyergost-8(14)-ene, which after recrystallization from chloroform–methanol formed small needles, m. p. 132·5—134°, $[\alpha]_D$ +10° (*c*, 1·95). Barton and Cox (*loc. cit.*) give m. p. 132°, $[\alpha]_D$ +11°.

3-*Ketoergost*-8(14)-*ene*.—The chromic acid solution (*ca.* 8N.) used as the oxidizing agent was prepared by dissolving pure chromium trioxide (66·7 g.) in water, adding concentrated sulphuric acid (53·3 c.c.), and diluting the mixture to 250 c.c. with water. A solution of 3β-hydroxyergost-8(14)-ene (5 g.) in AnalaR acetone (500 c.c.) was stirred vigorously at 30—35°. Chromic acid solution (10 c.c.) was added during 3 minutes, and after a further minute's stirring the excess of oxidant was destroyed by addition of a solution of sulphur dioxide in acetone. Saturated aqueous potassium carbonate was added, together with ether, and the acetone–ether layer separated. Drying and evaporation of this layer gave a product (4·2 g.) which was chromatographed on alumina (100 g.). Elution with benzene gave the ketone, which after recrystallization from ethanol–methanol formed platelets (3 g.), m. p. 128—130°, $[\alpha]_D$ +29° (*c*, 2·4). Barton and Cox (*loc. cit.*) record m. p. 129—130°, $[\alpha]_D$ +30° (*c*, 1·91).

Ergost-8(14)-*ene*.—The foregoing ketone (1 g.) was heated with hydrazine hydrate (7 c.c. of 60% w/v) and a solution of sodium (1·25 g.) in ethanol (25 c.c.) in an autoclave at 190—215° for 5 hours. The steroid was isolated with pentane and chromatographed on alumina (50 g.). Elution with pentane gave ergost-8(14)-ene (0·86 g.) which after recrystallization from ethyl acetate–methanol formed microscopic prisms, m. p. 79·5—81°, $[\alpha]_D$ +13° (*c*, 3·0). Heilbron, Spring, and Webster (*loc. cit.*) give m. p. 77—78°, $[\alpha]_D$ +11° (*c*, 1·0).

3β-*Hydroxyergost*-7-*ene* (γ-*Ergostenol*).—Difficulty was encountered in the preparation of the acetate of this sterol by hydrogenation of 3β-acetoxyergosta-7 : 22-diene in ethyl acetate solution with a platinum oxide catalyst owing to incomplete hydrogenation. However, use of a Raney nickel catalyst gave high and reproducible yields. A solution of the acetoxy-diene (4·4 g.) in ethyl acetate (350 c.c.) (kept at 30° initially in order to keep the solid in solution) was shaken with Raney nickel (1·5 g.) in hydrogen at atmospheric pressure until no more hydrogen was taken up (20 hours). The product was recrystallized from chloroform–methanol and ethyl acetate–methanol, to give 3β-acetoxyergost-7-ene (4·0 g.) as plates, m. p. 158—160°, $[\alpha]_D$ −3° (*c*, 4·1). Barton and Cox (*loc. cit.*) give m. p. 157—159°, $[\alpha]_D$ −4° (*c*, 1·9). Similar material was obtained by hydrogenation of ergosteryl acetate under similar conditions. 3β-Hydroxyergost-7-ene, prepared by alkaline hydrolysis of the acetate, formed needles, m. p. 149—150°, $[\alpha]_D$ −4° (*c*, 1·1). Barton and Cox (*loc. cit.*) give m. p. 148°, $[\alpha]_D$ −2° (*c*, 1·43).

3-*Ketoergost*-7-*ene*.—Oxidation of the foregoing sterol by the general chromic acid method gave this ketone as platelets from benzene, m. p. 158—159°, $[\alpha]_D$ +20° (*c*, 1·34) (Found : C, 84·4; H, 11·95. Calc. for $C_{28}H_{44}O$: C, 84·4; H, 11·6%). Barton and Cox (*loc. cit.*) give m. p. 159°, $[\alpha]_D$ +22° (*c*, 1·59).

Ergost-7-*ene*.—This was prepared by Kishner–Wolff reduction of the 3-ketone, as described for the preparation of ergosta-8(14)-ene. The hydrocarbon was purified by chromatography and by recrystallization from ethyl acetate–methanol. *Ergost*-7-*ene* exhibits "liquid crystal" formation when the m. p. is observed with crossed Nicol prisms, an anisotropic liquid forming at 83—84°, which becomes isotropic at 88—90°, $[\alpha]_D$ +0·5° (*c*, 1·15) (Found : C, 87·6; H, 12·8. $C_{28}H_{48}$ requires C, 87·4; H, 12·6%).

24-*Hydroxychol*-9(11)-*ene*.—Lithium aluminium hydride reduction of methyl chol-9(11)-enate (m. p. 65—66·5°, $[\alpha]_D$ +40° (*c*, 1·41)), kindly supplied by Dr. Heymann, Harvard University, in ether at 0° gave, after chromatographic purification, a nearly quantitative yield of 24-*hydroxychol*-9(11)-*ene*, as needles [from light petroleum (b. p. 40—60°)], m. p. 103—104°, $[\alpha]_D$ +44° (*c*, 0·97) (Found : C, 83·45; H, 11·6. $C_{24}H_{40}O$ requires C, 83·6; H, 11·7%).

Methyl 3α-hydroxychol-9(11)-enate (m. p. 105—108°) was kindly provided by Professor C. W. Shoppee.

3β-*Hydroxyergost*-14-*ene*.—A sample of the acetate, which had m. p. 113—114·5°, $[\alpha]_D$ +11° (*c*, 2·39), was kindly supplied by Dr. D. H. R. Barton. Hydrolysis and recrystallization from methanol gave the sterol as plates, m. p. 142—143·5°, $[\alpha]_D$ +21° (*c*, 1·05). Barton and Cox (*loc. cit.*) record m. p. 141°, $[\alpha]_D$ +22° (*c*, 1·26). Hart and Emerson (*J. Amer. Chem. Soc.*, 1932, **54**, 1070) give m. p. 141°, $[\alpha]_D$ +21·2°.

Spectroscopic Technique.—All spectra were run on a Grubb–Parsons single-beam spectrometer as described previously (Barnard, Fabian, and Koch, *J.*, 1950, 2442). A lithium fluoride prism was used for the 3·3-μ. region. The following linear slit-widths were employed : 0·09—0·11 mm. at 3·3 and at 6 μ., 0·18—0·45 mm. at 10—15 μ. The accuracy of the frequency measurements given in the Table is estimated as ±5 cm.$^{-1}$ at 3·3 μ., ±3 cm.$^{-1}$ at 6 μ., and ±2 cm.$^{-1}$ at 10—15 μ.

The water-vapour concentration in the spectrometer was reduced by means of continuous air circulation through an external tower of activated alumina in a closed system (built for us by Messrs. Air Control Installations Ltd.), whereupon only the stronger H_2O absorption peaks near 6 μ. remained conveniently defined for calibration purposes as shown in Fig. 3. The broad energy minimum on the spectral traces, superimposed on the water-vapour bands above 1700 cm.$^{-1}$, is due to the peculiar characteristics (interference fringes) of the Bell thermistor receiver employed as radiation detector.

No attempt was made to apply micro-methods in this work, but the primary focus in the radiation unit of the spectrometer, where a reduced image of the Nernst filament source is formed, was found very suitable for work on a semi-micro-scale. Fixed araldite-sealed rock-salt cells of the type previously described (Barnard, Fabian, and Koch, *loc. cit.*) were used, requiring about 0·1 c.c. of solution. Under

Infra-red frequencies for steroid compounds containing nuclear tri- and tetra-substituted double bonds.

Compound.	3000—3100 cm.$^{-1}$ (3·3 μ.). (CCl_4 soln.) (sh. = shoulder).	790—850 cm.$^{-1}$ (12 μ.). (CS_2 soln.)(<20% A = w., 20—50% A = m., >50% A = st.).	1620—1700 cm.$^{-1}$ (6 μ.). ($CHCl_3$ soln.).
Hydrocarbons:			
(*a*) [Cholestane]	—	[w. 795, w. 827]	—
(*b*) Cholest-4-ene	sh. 3040	st. 810	1657
(*c*) Cholest-5-ene	weak 3030	st. 797, st. 832	1667
(*d*) Ergost-7-ene	sh. 3013, sh. 3040	m. 795, st. 825	1666
(*e*) Cholest-8(9)-ene	—	w. 800, w. 833	—
(*f*) Ergost-8(14)-ene	—	w. 797	—
Ketones:			
(*g*) [Cholestanone]	—	[w. 808]	—
(*h*) Ergost-7-en-3-one	sh. 3013, sh. 3040	st. 827, st. 847	~1664
(*i*) Cholest-8(9)-en-3-one	—	w. 815	—
(*j*) Ergost-8(14)-en-3-one	—	w. 812	—
Alcohols:			
(*k*) [Cholestanol]	—	[w. 795, w. 842]	—
(*l*) Cholesterol	weak 3030	st. 800, m. 840 (CS_2 or Nujol)	1669
(*m*) 3β-Hydroxyergost-7-ene	sh. 3020, sh. 3040	m. 792, st. 828, m. 847 (Nujol)	1664
(*n*) 3β-Hydroxycholest-8(9)-ene	—	m. 802, m. 847 (Nujol)	—
(*o*) 3β-Hydroxyergost-8(14)-ene	—	m. 845 (Nujol)	—
(*p*) 3β-Hydroxyergost-14-ene	strong 3055	m. 797, m. 807, st. 825	1648
(*q*) 24-Hydroxychol-9(11)-ene	strong 3042	m. 827, m. 852	1644
Esters:			
(*r*) Methyl chol-9(11)-enate	moderate 3037	[not measured]	1643
(*s*) Methyl 3-hydroxychol-9(11)-enate	moderate 3037	[not measured]	1645

the standard conditions chosen for this work, a total of about 60 mg. of sample was applied over the three characteristic spectral regions approximately as follows: 5 mg. dissolved in 0·15 c.c. of carbon tetrachloride, path-length 1 mm., at 3·3 μ.; 25 mg. (15—20 mg. for compounds containing >C=O) in 0·15 c.c. of chloroform, path-length 1 mm., at 6 μ.; 20 mg. in 0·1 c.c. of carbon disulphide, path-length 0·25 mm., at 10—15 μ. If so desired, the samples could be recovered from each solution, and a total of about 35 mg. would then suffice for all measurements. After recovery from chloroform, however, great care must be taken to remove the last traces of this solvent which has intense bands near 3·3 and 13 μ.

Some steroid substances containing free hydroxyl groups are not sufficiently soluble for these standard conditions. At 3·3 μ., if the sample is virtually insoluble in carbon tetrachloride, rather thick pastes with perfluorokerosene (Dupont) give quite serviceable spectral curves; at 6 μ., the chloroform solution path-length can be increased to about 3 mm. with fair success or, in favourable cases, the double-bond peak can also be discerned in relatively thick Nujol mulls; at 10—15 μ. Nujol mulls are applied in a thickness giving roughly the same overall appearance of the spectral intensities near 10 μ. as that obtained with the standard carbon disulphide solutions (cf. Fig. 2, l_1 and l_2). The quality of the spectra obtainable from Nujol mulls varies from sample to sample, depending on the microscopic behaviour of the particular crystal structure on being ground to a powder.

Results and Discussion.

The Olefinic C–H *Stretching Region* (3·3 μ.).—As may be seen from Fig. 1 and the Table of frequencies, the steroid hydrocarbons, ketones, and alcohols containing nuclear tetrasubstituted ($\Delta^{8(9)}$- or $\Delta^{8(14)}$-)double bonds resemble their saturated analogues in failing to give rise to selective absorption between 3000 and 3100 cm.$^{-1}$, reflecting the absence of olefinic C–H groups (cf. Jones *et al.*, *loc. cit.*, 1948). On the other hand, all nuclear trisubstituted ethylenic compounds exhibited such selective absorption to a greater or lesser degree (Fig. 1 and Table). The $\Delta^{9(11)}$- and Δ^{14}-groups gave quite well-defined bands, cholest-5-ene (*c*) and the corresponding alcohol, cholesterol (*l*), displayed small peaks, and the Δ^{4}- and Δ^{7}-compounds were just characterized by shoulders on the slope of the strong saturated C–H absorption; the shoulder was apparently double in the latter case. Only a single band or shoulder might perhaps be expected to arise in all these spectra, but both Fox and Martin (*loc. cit.*) and we have observed a doublet similar to that found for the Δ^{7}-compounds also in methyl*cyclo*hexene, presumably resulting from vibrational interaction of the single olefinic C–H bond with the neighbouring CH_3 group.

Clearly, the appearance of the 3000—3100-cm.$^{-1}$ spectral region examined under sufficient dispersion may help to distinguish between the tetrasubstituted ethylenic type on one hand,

and the trisubstituted (as well as other lesser substituted types) on the other. Some intensity differentiation between the various trisubstituted compounds also appears to take place and may perhaps be specific (see also below). Certain reserve must, however, be exercised in drawing structural conclusions on an unknown compound from this spectral region alone; *absence* of selective absorption may conceivably result from excessive weakness of the characteristic C–H band in a given case, and the *presence* of a band or shoulder must be checked against possible strong absorption near 1550 cm.$^{-1}$ which might give rise to a detectable first overtone. Unfortunately it is not possible to improve the prominence of weak bands by increasing sample concentration or optical resolution still further, as the persistence and effective resolution of these bands is limited by the overlapping of the adjoining relatively much more intense absorption region of saturated C–H just below 3000 cm.$^{-1}$.

The Olefinic C–H *Out-of-plane Bending Region* (10—15 μ.).—Similarly to the above, the presence or otherwise of olefinic C–H bonds in the steroid nucleus can frequently be recognized from the 10—15-μ. spectral region. All the trisubstituted ethylenic derivatives reported here displayed moderate or strong absorptions between 790 and 850 cm.$^{-1}$, where the tetrasubstituted examples, whether hydrocarbon, ketone, or alcohol, only gave relatively weak skeletal frequencies (Fig. 2 and third column of the table). The closest approach between the two types of unsaturation as examined under approximately standardized concentration conditions was that of the trisubstituted ethylenic 24-hydroxycholest-9(11)-ene (*g*) which exhibited barely 40% absorption at 827 cm.$^{-1}$ and the tetrasubstituted 3β-hydroxycholest-8(9)-ene (*n*) which apparently (in Nujol) absorbed nearly 25% at 802 and 847 cm.$^{-1}$. It may also be noted here that the only substance giving rise to a moderately strong band between 650 and 700 cm.$^{-1}$ (the characteristic region of nuclear disubstituted double bonds) was cholest-4-ene (*b*).

With one exception (*b*), all the trisubstituted double-bond groups displayed *two* moderately strong bands instead of the single frequency normally expected for such systems at 790—850 cm.$^{-1}$. It may be that the true single frequency of the out-of-plane bending vibration of the olefinic C–H bond lies very near a weak skeletal vibration frequency of the same symmetry class which becomes greatly intensified through resonance interaction, so that two strong bands result. Such an explanation would be in accord with the weak bands generally observed in this spectral region for saturated and tetrasubstituted ethylenic steroid compounds.

The characteristic twin olefinic frequencies of cholesterol found at 800 and 840 cm.$^{-1}$ in either carbon disulphide solution (l_1) or the crystalline solid (l_2) are closely similar to those reported in the literature (*loc. cit.*). In view of the marked changes which occur in this spectral region of crystalline cholesterol, on cooling to very low temperatures (without, however, affecting the strong doublet) (Haines *et al.*, *loc. cit.*), the close correspondence between the absorption curves obtained at room temperature for the carbon disulphide solution and for the crystalline solid seems of interest. The physical condition of the steroid sample appears to have no important influence on the position or intensity of the diagnostic bands. Much more marked spectral changes at 790—850 cm.$^{-1}$ have been found to differentiate the various physical states of *cis*- and *trans*-polyisoprenes severally (Saunders and Smith, *loc. cit.*).

Diagnostic application of the C–H out-of-plane bending region has already been made in the structurally related pentacyclic triterpene group. Thus, two of us (quoted by Ames, Halsall, and Jones, *J.*, 1951, 450) inferred from the low absorption intensity of β-amyrene-III between 10 and 15 μ. that the double bond of this unsaturated hydrocarbon must be in a tetrasubstituted position. Similarly, Günthard (quoted by Dietrich, Meyer, Jeger, and Ruzicka, *Helv. Chim. Acta*, 1950, **33**, 672, 711) found in the presence of a trisubstituted double bond in soyasapogenol-C reflected by a moderately strong band at 815 cm.$^{-1}$, and double-bond isomerization in a pentacyclic hydrocarbon was significantly correlated with the disappearance of a band at 800 cm.$^{-1}$ to give a high-transmission spectral curve. It would seem that extreme weakness of absorption in the 790—850-cm.$^{-1}$ range, examined at sufficient path-length or concentration, is a reliable criterion of the *absence* of trisubstituted double-bond groups; that bands of intermediate strength as observed in 3β-hydroxycholest-8(9)-ene or 24-hydroxycholest-9(11)-ene may be diagnostically unreliable; and that strong bands as in cholesterol and cholest-4-ene give a fairly safe indication of the *presence* of such groups. Preliminary observations on disubstituted ethylenic compounds in the sterol group also lead us to conclude that weak absorption throughout the range 10—15 μ. may be regarded as proof of the absence of any but tetrasubstituted olefinic linkages in the steroid molecule.

The C=C *Stretching Region* (6 μ.).—A reproduction of the actual spectral energy traces as obtained on the automatic recorder at 6 μ. is shown in Fig. 3 so as to afford an indication of the

slight degree of interference by water-vapour absorption and the relative prominence of the characteristic double-bond peaks which can be observed. The frequency position of these bands is given in the last column of the table. It was found that all of the trisubstituted ethylenic derivatives, including one unconjugated ketone (*h*) and two esters (*r* and *s*), displayed weak or moderate bands at 1640—1670 cm.$^{-1}$, whereas none of the saturated or tetrasubstituted olefinic compounds gave any such absorption under comparable conditions. Jones *et al.* (*loc. cit.*, 1950) similarly observed double-bond frequencies for ten Δ^5- (1669—1672), one Δ^7- (1664), four Δ^{14}- (1646—1648) and three Δ^{16}- (1621—1630 cm.$^{-1}$) unsaturated trisubstituted ethylenic molecules, but they failed to observe such a frequency in five different $\Delta^{9(11)}$- unsaturated steroids containing one or more carbonyl groups which render its detection rather difficult. The tetrasubstituted ethylenic cholest-8(14)-enol was also found transparent by these authors.

The diagnostically significant intensity differentiation observed between tri- and tetrasubstituted ethylenic steroids, in which the double-bond stretching vibration appears weakly allowed and forbidden respectively in the infra-red, is in accord with symmetry considerations. It seems unlikely that any of the bands found in this region should merely represent overtones of the C–H bending vibrations at 790—850 cm.$^{-1}$ rather than genuine double-bond stretching frequencies. Cholest-4-ene (*b*), for instance, displays a particularly intense characteristic frequency at 810 cm.$^{-1}$ which corresponds to a first overtone frequency at or below 1620 cm.$^{-1}$, whereas the observed 6-μ. absorption actually occurs at 1657 cm.$^{-1}$. Again, ergost-7-ene (*d*) exhibits a strong band at 825 cm.$^{-1}$ with a potential overtone normally expected at or below 1650 cm.$^{-1}$ so that the rather intense 1666-cm.$^{-1}$ peak is much more reasonably assigned to the stretching vibration of the double bond.

Only the Δ^5- and Δ^7-compounds give characteristic frequencies in the usual trialkylethylene range of 1660—1675 cm.$^{-1}$. These two appear too close together to permit unequivocal differentiation, although the Δ^5 frequencies seem consistently higher by a few wave-numbers. The Δ^{14}- and $\Delta^{9(11)}$-compounds (*f*) and (*q*, *r*, *s*) are similarly difficult to distinguish from each other on the basis of their double-bond vibration frequencies alone, but they are sharply differentiated from the other trialkylethylenes by the abnormal location of these bands below 1650 cm.$^{-1}$. On account of the relatively large shifts involved, we believe that changes from the normal stretching-force constants of trisubstituted double bonds must be at least partly responsible for the effect, rather than only unspecific changes in the effective mass of the vibrating groups. This supposition is supported by the following considerations.

The Effect of Steric Strain.—Monocyclic five-membered ring compounds containing one or more unsaturated carbon atoms, such as *cyclo*pentanone or *cyclo*pentene, are subject to appreciable steric strain in Baeyer's sense. Extrapolating the quantum-mechanical treatment of *cyclo*propane and *cyclo*butane by Coulson and Moffitt (*Phil. Mag.*, 1949, **40**, 1; cf. Förster, *Z. physikal. Chem.*, 1939, *B*, **43**, 58), such strain should be taken up by $s : p$ hybridization ratio changes in the carbon σ-valency orbitals and by formation of " bent " σ-bonds, resulting in all exocyclic bonds becoming slightly strengthened as well as the cyclic bonds being weakened. The theory accounts for the anomalous C–H bond force constants which are in fact observed in both *cyclo*propane and *cyclo*butane (*loc. cit.*), and corresponding force-constant changes may therefore be expected to occur also in strained unsaturated five-membered rings. The reality of such changes appears to be reflected in the unusually high stretching vibration frequencies of the olefinic C–H bonds in *cyclo*pentene (Kohlrausch and Seka, *Ber.*, 1936, **69**, 729; Kohlrausch, Seka, and Tramposch, *ibid.*, 1942, **75**, 1385) and of the carbonyl bond in *cyclo*pentanone (Hartwell, Richards, and Thompson, *J.*, 1948, 1036), as well as in the abnormally low C=C vibration frequencies of *cyclo*pentene and related hydrocarbons (Kohlrausch *et al.*, *loc. cit.*). We may conclude that the anomalous 1648-cm.$^{-1}$ frequency of 3β-hydroxyergost-14-ene (*p*) forms part of a more general phenomenon comprising also the related anomalies of Δ^{16}- and of 17-keto-steroids (Jones *et al.*, *loc. cit.*), and that it corresponds to a reduction in bond-stretching force constant resulting from σ-bond strain in ring D of the steroid nucleus. This interpretation receives further support from the unusually high (as well as abnormally intense) olefinic C–H stretching frequency of the Δ^{14}-molecule (*p*) at 3055 cm.$^{-1}$.

The lowered C=C stretching frequency of $\Delta^{9(11)}$-compounds (*q*, *r*, and *s*) finds a significant parallel in a similar anomaly of Δ^{11}-derivatives (Jones *et al.*, *loc. cit.*, 1950, and our observations). In both cases, space models suggest the possibility of appreciable steric strain resulting from *trans*-fusion of *cyclo*pentane and *cyclo*hexene rings. Related anomalies should also occur in $\Delta^{8(9)}$- and $\Delta^{8(14)}$-steroids but cannot be observed in the infra-red where the double-bond vibration is inactive. An interesting analogy seems to be provided, however, by the fusion of a *cyclo*butane to a *cyclo*hexene ring as in α-pinene, resulting in a reduction of the normal 1675-cm.$^{-1}$

double-bond stretching frequency of isomeric 1-methyl-4-*iso*propenyl*cyclo*hexene to 1656 cm.$^{-1}$ (A.P.I., *loc. cit.*).

If the apparent steric strain in both $\Delta^{9(11)}$- and Δ^{14}-steroids is assumed to be responsible also for a reduction in the strength of the π-bond of these ethylenic linkages, rather than for changes of σ-orbital hybridization alone, then the observed intensities of the adjacent C–H bond-vibration frequencies can be better understood. In the presence of σ-bond strain alone, we may expect a slight frequency increase and probably a negligible change of infra-red absorption intensity (cf. comparative extinction coefficients of sp^2 and sp^3 C–H bonds given by Fox and Martin, *loc. cit.*). For π-bond strain, on the other hand, involving reduced π-orbital overlap, the C–H bond frequency should remain essentially unchanged but its intensity might well be markedly affected on account of the greater residual electron density now remaining on the unsaturated carbon atom. Fig 1 illustrates the fact that a remarkable intensification of the olefinic C–H absorption occurs in 3β-hydroxyergost-14-ene (*p*) and to a lesser extent also in the $\Delta^{9(11)}$-compounds (*q*, *r*, and *s*).

It has been tacitly assumed in the foregoing that the C=C stretching vibration is entirely localized in the double bond and that interaction with neighbouring groups can be, as customary, neglected to a first approximation. This might no longer be true in the presence of steric strain, but the reported unusual infra-red absorption properties of the essentially localized olefinic C–H bond vibration strongly support the view that some real change from the normal electronic structure does occur at the unsaturated carbon atom.

Isolated double bonds in rings A or B of the steroid nucleus evidently do not create appreciable steric strain. Δ^5- and Δ^7-Groups display normal vibrational behaviour, and although the 1657-cm.$^{-1}$ frequency of cholest-4-ene (*b*) seems slightly low, it is not paralleled by any corresponding anomaly in the absorption of a Δ^2-group in the same ring (Jones *et al.*, *loc. cit.*, and forthcoming paper in this series).

We are greatly indebted to Messrs. Glaxo Laboratories Ltd. for a generous gift of α-dihydroergosterol and for financial assistance (to P. B.), and to Dr. D. H. R. Barton, Dr. H. Heymann, and Prof. C. W. Shoppee for samples specified above. Further, thanks are due to Prof. E. R. H. Jones, F.R.S., for his constant encouragement and interest in this work, and to Dr. G. Gee for permission to carry out the spectroscopic measurements in the laboratories of the British Rubber Producers' Research Association. One of us (G. W. W.) thanks the Department of Scientific and Industrial Research for a maintenance grant.

THE UNIVERSITY, MANCHESTER, 13. [*Received, March 7th*, 1951.]

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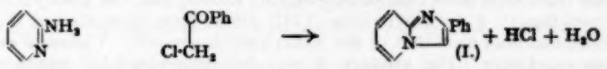
533. *Preparation of Pyridinobenziminazole and its Derivatives.*

By NEIL CAMPBELL and E. B. MCCALL.

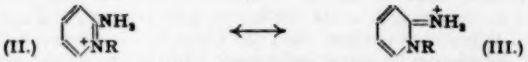
2-Aminopyridine and its 3- and 5-substituted derivatives condense with 2-chloro*cyclo*hexanone to form 4 : 5 : 6 : 7-tetrahydropyridino(1′ : 2′-1 : 2)-benziminazoles. The unsubstituted tetrahydro-compound on dehydrogenation yields pyridino(1′ : 2′-1 : 2)benziminazole.

TSCHITSCHIBABIN (*Ber.*, 1924, **57**, 2092; 1925, **58**, 1704) showed that 2-aminopyridine combines with α-halogenated ketones or esters to give pyridino(1′ : 2′-1 : 2)glyoxalines, the halogen attacking the ring-nitrogen atom rather than the primary amino-group. In agreement with this and of relevance to the present work is the observation that ω-chloroacetophenone and 2-aminopyridine yield 4-phenylpyridino(1′ : 2′-1 : 2)glyoxaline (I) whose structure was determined by Schmid and Bangler (*Ber.*, 1925, **58**, 1971; 1926, **59**, 1360). It is doubtful if such



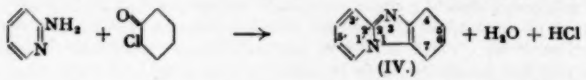
reactions are as simple as represented, and it is pertinent to mention that the long-chain alkyl iodides give with 2-aminopyridine in cymene a mixture containing 75—85% of the strongly basic 1-alkyl-2-pyridone imine and 15—25% of the isomeric 2-alkylaminopyridine (Sharp, *J.*, 1939, 1855). Even here, however, the ring nitrogen is the main centre of attack. Theoretically this is to be expected since the cation (II; R = CH_2·COPh, etc.) resulting from the quaternary-salt formation can resonate with the form (III) (cf. Mann and Watson, *J. Org.*

Chem., 1948, **13**, 502). Attack at the primary amino-group would give rise to products without such resonance stabilisation.



(II.) (III.)

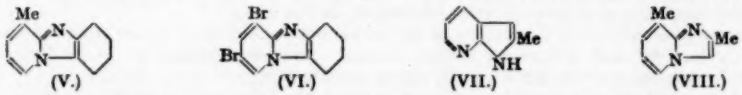
It would be expected from such considerations that 2-aminopyridine and 2-chloro*cyclo*hexanone would give 4 : 5 : 6 : 7-tetrahydropyridino(1′ : 2′-1 : 2)benziminazole (IV) and not 6 : 7 : 8 : 9-tetrahydro-2-carboline as claimed in an I.G. Farbenindustrie patent (B.P. 360,027;



(IV.)

cf. *Chem. Abstracts*, 1932, **26**, 3514). It was therefore decided to reinvestigate the reaction, employing not only 2-aminopyridine but also suitably substituted derivatives.

2-Aminopyridine and 2-chloro*cyclo*hexanone condense to give a product apparently identical with that obtained by the German workers except that it crystallises from organic solvents as the monohydrate, $C_{11}H_{12}N_2,H_2O$, m. p. 56—58°, a fact not mentioned in the patent. The hydrate



(V.) (VI.) (VII.) (VIII.)

is readily dehydrated *in vacuo* to give the anhydrous base, m. p. 95—96° (*A*). 2-Amino-3-methylpyridine and 2-amino-3 : 5-dibromopyridine in the same way give respectively 4 : 5 : 6 : 7-tetrahydro-3′-methyl- (V; as dihydrate) and -3′ : 5′-dibromo-pyridino(1′ : 2′-1 : 2)benziminazole (VI) the structures of which are thus unambiguously determined.

Comparison of the ultra-violet absorption spectra of the 3′ : 5′-dibromo-compound and the product *A* shows great similarity (Fig. 1) and provides evidence that the cyclisation of 2-aminopyridine and 2-chloro*cyclo*hexanone occurs at the two nitrogen atoms to give a pyridinobenziminazole derivative. The two absorption curves show that in the wave-length region 230—400 mμ. the two substances exhibit almost identical absorption, the only difference being the expected displacement of 10—20 mμ. towards the longer wave-lengths by the bromine auxochromes (see Braude, *Ann. Reports*, 1945, **42**, 124). The condensation product of 2-amino-5-bromopyridine and 2-chloro*cyclo*hexanone likewise has a similar absorption curve with maxima at 245 mμ. (log ε 4·36), 290 mμ. (log ε 3·63), and 325 mμ. (log ε 3·70), suggesting that it is 5′-bromo-4 : 5 : 6 : 7-tetrahydropyridino(1′ : 2′-1 : 2)benziminazole. Here also displacement of the absorption maxima by bromine is obvious. Final conclusions cannot be drawn unless these absorption spectra are shown to be distinctly different from those of 6 : 7 : 8 : 9-tetrahydro-2-carboline. This compound not having been prepared, its absorption curve is not known but,

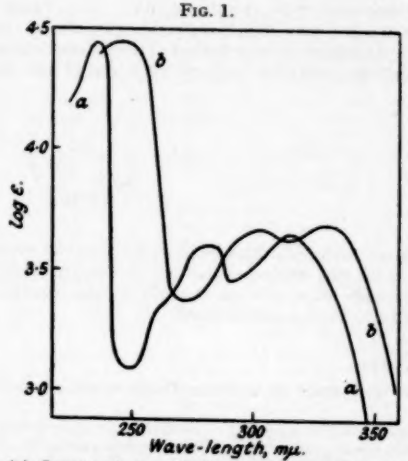
(IX.) (X.)

since the methylene groups of the reduced ring contribute little or nothing to the absorption (see R. N. Jones, *Chem. Reviews*, 1943, **32**, 21), the absorption of the tetrahydrocarboline will probably be essentially the same as that of pyridino(2′ : 3′-2 : 3)pyrrole or its alkyl derivatives. Fig. 2 shows that the spectra of tetrahydropyridino(1′ : 2′-1 : 2)benziminazole and 5-methylpyridino(2′ : 3′-2 : 3)pyrrole (VII) are different, but it is not legitimate to draw structural conclusions from these data since control experiments showed that the absorption spectrum of 4 : 3′-dimethylpyridino(1′ : 2′-1 : 2)glyoxaline (VIII) differs more from that of the tetrahydropyridinobenziminazole than from that of the methylpyridinopyrrole. Chemical evidence, however, was more conclusive. The product *A* was dehydrogenated by means of palladised charcoal at 300° (cf. Horning, Horning, and Walker, *J. Amer. Chem. Soc.*, 1948, **70**, 3935) to give a product with properties similar to those of pyridino(1′ : 2′-1 : 2)benziminazole previously synthesised by Morgan and Stewart (*J.*, 1938, 1292; 1939, 1057).

2-2′-Pyridylamino*cyclo*hexanone (IX) is stated to be prepared from 2-sodioaminopyridine and 2-chloro*cyclo*hexanone (B.P. 360,027). We have found that it is more conveniently obtained by boiling 2-aminopyridine and 2-chloro*cyclo*hexanone in ethanol containing sodium

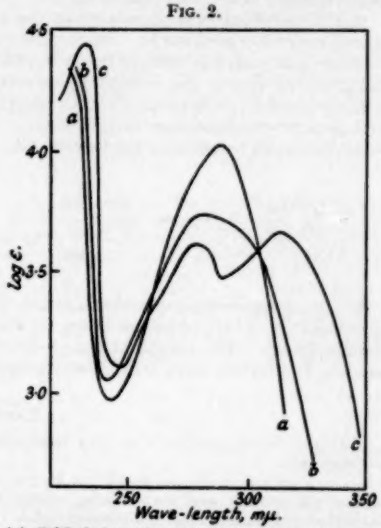
carbonate although the yields are only 30%. To ensure that the product is not 1-2′-ketocyclohexyl-2-pyridone imine (X), its ultra-violet absorption spectrum was measured and compared with those of 2-methylaminopyridine and 1-methyl-2-pyridone imine, recently measured by Anderson and Seeger (*J. Amer. Chem. Soc.*, 1949, **71**, 340). Fig. 3 shows that in the above

FIG. 1.



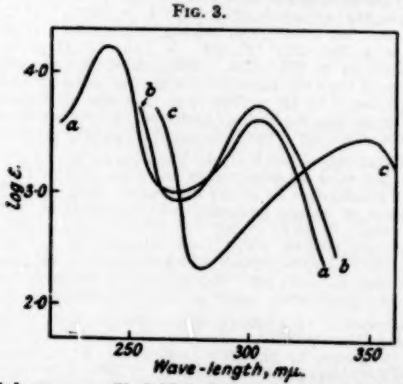
(*a*) *Compound A.* (*b*) 3′ : 5′-*Dibromo*-4 : 5 : 6 : 7-*tetrahydropyridino*(1′ : 2′-1 : 2)*benziminazole.*

FIG. 2.



(*a*) 5-*Methylpyridino*(2′ : 3′-2 : 3)*pyrrole.* (*b*) 4 : 3′-*Dimethylpyridino*(1′ : 2′-1 : 2)*glyoxaline.* (*c*) 4 : 5 : 6 : 7-*Tetrahydropyridino*(1′ : 2′-1 : 2)*benziminazole.*

FIG. 3.

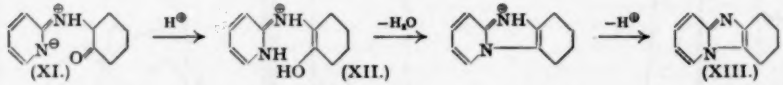


(*a*) 2-2′-*Pyridylaminocyclohexanone.* (*b*) 2-*Methylaminopyridine.* (*c*) 1-*Methyl*-2-*pyridone imine.*

reaction the halogen atom has reacted with the amino-group of 2-aminopyridine to give the pyridylamino*cyclo*hexanone. This was confirmed by the chemical properties of the product. With boiling ethanolic sodium hydroxide it gave no ammonia, thus showing the absence of an imino-group; the only product isolated was 2-2′-pyridylamino*cyclo*hexanol, which was also formed when aluminium *iso*propoxide in *iso*propanol was used. Presumably in the first instance some sodium ethoxide was formed and this catalysed a hydrogen transfer of the Meerwein–Ponndorf type. Reduction by Huang-Minlon's modification of the Wolff–Kishner

method (*J. Amer. Chem. Soc.*, 1946, **68**, 2487) gave 2-*cyclo*hexylaminopyridine, identical with the product formed by condensation of 2-bromopyridine and *cyclo*hexylamine. Clemmensen reduction cleaved the molecule, and 2-aminopyridine was identified as one of the products. Similar cleavages of amino-ketones by the Clemmensen method have been reported (von Braun and Weissbach, *Ber.*, 1929, **62**, 2416).

2-2′-Pyridylamino*cyclo*hexanone in the presence of hydrogen ions readily yields the tetrahydropyridinobenziminazole. Since protons add preferentially to the ring-nitrogen atom, a plausible mechanism is outlined below in which proton adds to the polar form (XI). The product (XII) undergoes ring-closure by dehydration, and the free base (XIII) is liberated by alkali. It is somewhat difficult to account for the observation that some of the substituted 2-aminopyridines with 2-chloro*cyclo*hexanone in the presence of sodium carbonate undergo ring-closure and no pyridylamino*cyclo*hexanone can be isolated.



It would appear from the above results that 2-aminopyridine and 2-chloro-ketones can yield pyridino(1′ : 2′-1 : 2)glyoxalines either by attack at the ring-nitrogen atom (see p. 2411) or at the 2-amino-group. The course taken evidently depends *inter alia* on the pH of the reaction mixture, but further work is necessary before a full assessment can be made.

Experimental.

All ultra-violet absorption spectra measurements were made on a Hilger Barfit medium quartz spectrograph.

4 : 5 : 6 : 7-*Tetrahydropyridino*(1′ : 2′-1 : 2)*benziminazole*.—The patented method (*loc. cit.*) gave a 33% yield of the product, and the following modified procedure is preferable. 2-Chloro*cyclo*hexanone (8 g.) was added dropwise to boiling 2-aminopyridine (11·4 g., 2 mols.), and the mixture was boiled gently for a further 15 minutes. The solution was made alkaline with sodium hydroxide and steam-dstilled until the distillate was free from aminopyridine. The residue was extracted with benzene, and the dried extract (Na_2SO_4) on evaporation gave a syrup which distilled at 170—180°/12 mm. and slowly crystallised, m. p. 45—65° (yield 7·4 g., 65%). Recrystallisation from ether–light petroleum gave **4 : 5 : 6 : 7-*tetrahydropyridino*(1′ : 2′-1 : 2)*benziminazole monohydrate***, m. p. 56—58° (Found: C, 69·7; H, 7·3; N, 15·2%; *M*, 174. $C_{11}H_{12}N_2,H_2O$ requires C, 69·5; H, 7·4; N, 14·7%; *M*, 190), with absorption maxima at 235 mμ. (log ε 4·45), 281 mμ. (log ε 3·62), and 316 mμ. (log ε 3·66). It gives a *picrate* (yellow needles from ethanol–acetic acid), m. p. 260—261° (Found: N, 17·4. $C_{17}H_{15}O_7N_5$ requires N, 17·5%), and a methiodide (colourless needles), m. p. 256—258°. When dried over phosphoric oxide for 48—72 hours and finally in a high vacuum, it loses the theoretical weight of water to form the anhydrous substance, m. p. 95—96°, which on exposure to air rapidly reverts to the monohydrate. Examination on the heating-stage microscope showed that the hydrate melts to a cloudy liquid which does not clear until the water of crystallisation distils off at 120°; the clear liquid on cooling crystallises in the anhydrous form.

4 : 5 : 6 : 7-*Tetrahydro-3′-methylpyridino*(1′ : 2′-1 : 2)*benziminazole*.—2-Amino-3-methylpyridine (5 g.) and 2-chloro*cyclo*hexanone (7·7 g., 1·25 mols.) were boiled in ethanol (30 ml.) for 28 hours, and the solvents distilled off until crystallisation of the hydrochloride, m. p. 235—237°, commenced. The free base was obtained by addition of sodium hydroxide and separated in long needles from water as the *dihydrate*, m. p. 60—61° (Found: C, 64·3; H, 8·0; N, 12·7. $C_{13}H_{14}N_2,2H_2O$ requires C, 64·8; H, 8·2; N, 12·6%). It forms a *picrate* (yellow plates from ethanol), m. p. 155—158° (Found: N, 16·9. $C_{18}H_{17}O_7N_5$ requires N, 17·1%). The substance is also obtained in 48% yield by boiling the reactants without a solvent. Distillation *in vacuo* gave the anhydrous compound, m. p. 85—87°, b. p. 230—240°/13 mm., which when crystallised from water gave the dihydrate, m. p. 52—54°.

5′-Bromo-* and *3′ : 5′-Dibromo*-4 : 5 : 6 : 7-*tetrahydropyridino*(1′ : 2′-1 : 2)*benziminazole*.**—2-Amino-3 : 5-dibromopyridine (5 g.) and 2-chloro*cyclo*hexanone (4 g., 1·5 mols.) were boiled in ethanol (30 ml.) for 26 hours and worked up as above to give ***3′ : 5′-dibromo*-4 : 5 : 6 : 7-*tetrahydropyridino*(1′ : 2′-1 : 2)-*benziminazole, which crystallised from light petroleum (b. p. 100—120°) in needles (2·4 g., 37%), m. p. 159—160° (Found: C, 40·5; H, 3·45; N, 8·4; Br, 47·9. $C_{11}H_{10}N_3Br_2$ requires C, 40·0; H, 3·1; N, 8·5; Br, 48·4%). It shows absorption maxima at 245 mμ. (log ε 4·45), 290 mμ. (log ε 3·68), and 325 mμ. (log ε 3·70), and forms a *picrate* (yellow needles from ethanol–acetic acid), m. p. 167—169° (Found: N, 12·5. $C_{11}H_{10}N_3Br_2,C_6H_3O_7N_3$ requires N, 12·4%). 2-Amino-5-bromopyridine similarly gave ***5′-bromo*-4 : 5 : 6 : 7-*tetrahydropyridino*(1′ : 2′-1 : 2)*benziminazole*** [prisms from light petroleum (b. p. 100—120°)] (73%), m. p. 148—149° (Found: C, 52·8; H, 4·4; N, 10·3; Br, 31·2. $C_{11}H_{11}N_3Br$ requires C, 52·6; H, 4·40; N, 11·2; Br, 31·8%), which has absorption maxima at 245 mμ. (log ε 4·45), 302 mμ. (log ε 3·68), and 332 mμ. (log ε 3·70). It forms a hydrochloride, m. p. 260—262°, and a *picrate* (yellow needles from glacial acetic acid), m. p. 264—265° (decomp.) (Found: N, 13·7. $C_{11}H_{11}N_2Br,C_6H_3O_7N_3$ requires N, 14·6%). The monobromo-compound was also obtained in 73% yield without the use of a solvent.

4 : 5 : 6 : 7-*Tetrahydro-5′-nitropyridino*(1′ : 2′-1 : 2)*benziminazole*.—2-Amino-5-nitropyridine (3·0 g.) and 2-chloro*cyclo*hexanone (2·9 g., 1 mol.) were mixed and the temperature was gradually raised until at 120—130° a vigorous reaction commenced. After this had subsided the mixture was kept at 130° for 2 hours. The black product was dissolved in boiling dilute hydrochloric acid (25 ml.) (charcoal), and the solution filtered. Neutralisation with sodium hydroxide gave the *nitro*-compound, which crystallised in orange-yellow needles from ethanol (charcoal) (1·4 g., 30%), m. p. 214—215° (Found: C, 60·6; H, 5·1; N, 18·5. $C_{11}H_{11}O_2N_3$ requires C, 60·8; H, 5·1; N, 19·3%). Its *picrate* formed yellow needles (ethanol–acetic acid), m. p. 216—219° (decomp.) (Found: N, 18·2. $C_{11}H_{11}O_2N_3,C_6H_3O_7N_3$ requires N, 18·8%).

Preparation of Pyridino(1′ : 2′-1 : 2)*benziminazole*.—4 : 5 : 6 : 7-Tetrahydropyridino(1′ : 2′-1 : 2)benziminazole (1 g.) and palladised charcoal (0·5 g.) were heated in a metal-bath at 300° for 6 hours and the residue was thoroughly extracted with boiling acetone. Evaporation of the solvent gave a solid (0·9 g.) which was dissolved in benzene (40 ml.), and the solution was passed down a column of alumina (24″ × 1″). Development of the column with benzene gave two zones: a lower, strongly fluorescent band of unchanged material, and an upper, pale green band which on extraction yielded pyridino(1′ : 2′-1 : 2)benziminazole (elongated prisms from benzene), m. p. 176—177° (lit., 178°) (Found: C, 78·5; H, 4·6; N, 17·1. Calc. for $C_{11}H_8N_2$: C, 78·5; H, 4·8; N, 16·7%).

Preparation of Substituted Pyridino(1′ : 2′-1 : 2)*glyoxalines*.—Bromoacetone and 2-aminopyridine gave a 10% yield of 4-methylpyridino(1′ : 2′-1 : 2)glyoxaline (Tschitschibabin, *Ber.*, 1926, **59**, 2054), b. p. 134—136°/15 mm. (Found: C, 71·9; H, 6·8; N, 21·3. Calc. for $C_9H_8N_2$: C, 72·7; H, 6·1; N, 21·2%). In view of the small yield the following modified procedure was adopted for the substituted derivatives. 2-Amino-3-methylpyridine (5 g.), bromoacetone (6·3 g., 1 mol.), and ethanol (30 ml.) were boiled until the smell of the bromoacetone could scarcely be detected (*ca.* 25 hours). Evaporation of the ethanol and addition of ether gave the 4 : 3′-dimethylpyridinoglyoxaline hydrobromide as a pale yellow mass, m. p. 248—250° (8·4 g.), which with sodium hydroxide gave the free *base* (needles from water or light petroleum) (4 g.), m. p. 42—45° (Found: C, 61·7; H, 7·5; N, 16·4. $C_9H_{10}N_2,1\frac{1}{2}H_2O$ requires C, 62·4; H, 7·6; N, 16·2%). It forms a *picrate* (yellow prisms from ethanol–acetic acid), m. p. 192—194° (Found: N, 18·8. $C_{15}H_{13}O_7N_5$ requires N, 18·7%).

2-Amino-3 : 5-dibromopyridine (5 g.) and bromoacetone (2·8 g.) in ethanol (30 ml.), when boiled for 40 hours, gave 3′ : 5′-dibromo-4-methylpyridino(1′ : 2′-1 : 2)glyoxaline hydrobromide, m. p. >300°, which with alkali afforded the *base* [needles from light petroleum (b. p. 100—120°)] (3·9 g.), m. p. 144—145° (Found: N, 9·7; Br, 55·0. $C_9H_6N_2Br_2$ requires N, 9·7; Br, 55·1%). This forms a *picrate* (yellow prisms from ethanol–acetic acid), m. p. 198—200° (Found: N, 13·3. $C_9H_6N_2Br_2,C_6H_3O_7N_3$ requires N, 13·5%). 2-Amino-5-bromopyridine (5 g.) similarly gave 5′-bromo-4-methylpyridinoglyoxaline hydrobromide (4·9 g.), m. p. 210—220°, 2·3 g. of which gave the *base* (1·2 g.) as needles [from light petroleum (b. p. 60—80°)], m. p. 102—103° (Found: C, 45·3; H, 3·6; N, 12·4; Br, 38·6. $C_9H_7N_2Br$ requires C, 45·5; H, 3·3; N, 13·3; Br, 37·9%); the *picrate* formed yellow needles (from ethanol–acetic acid), m. p. 226—228° (decomp.) (Found: N, 16·2. $C_9H_7N_2Br,C_6H_3O_7N_3$ requires N, 15·9%). 2-Amino-5-nitropyridine (3 g.) gave 4-methyl-5′-nitropyridinoglyoxaline hydrobromide (3 g.), m. p. >300°, which quantitatively gave the *base* (yellow needles from ethanol), m. p. 197—199° (slight decomp.) (Found: C, 54·0; H, 3·8; N, 23·2. $C_9H_7O_2N_3$ requires C, 54·2; H, 4·0; N, 23·7%). This gave a *picrate* as yellow plates (ethanol–acetic acid), m. p. 181—184° (decomp.) (Found: N, 20·2. $C_{14}H_{10}O_9N_6$ requires N, 20·7%).

2-Aminopyridine (3·1 g.) and ω-chloroacetophenone (5 g.) were boiled in ethanol (30 ml.) for 28 hours (cf. Tschitschibabin, *Ber.*, 1926, **59**, 2051; Schmid and Bangler, *loc. cit.*). Evaporation of the solvent gave an oil and a small quantity of slender needles, m. p. 114—116°, probably the *4-phenylpyridinoglyoxaline hydrochloride* (Found: C, 58·2; H, 5·1; N, 10·2; Cl, 13·1. $C_{13}H_{10}N_2,HCl,2H_2O$ requires C, 58·5; H, 5·7; N, 10·2; Cl, 13·3%). The oil, with sodium hydroxide, gave the 4-phenyl-base as a brown oil which slowly solidified and was crystallised from ethanol; it had m. p. 134—136° (lit., 135·5° and 140°) (Found: C, 80·0; H, 5·3. Calc. for $C_{13}H_{10}N_2$: C, 80·4; H, 5·2%). It gives a *picrate* as yellow needles (from glacial acetic acid), m. p. 228—229° (decomp.) after sintering at 200° (Found: N, 17·6, 15·4. $C_{19}H_{13}O_7N_5$ requires N, 16·6%).

2-Amino-3-methylpyridine (3 g.) and ω-chloroacetophenone (7·5 g.) were fused together, and the melt temperature gradually raised to 130°, whereupon a vigorous reaction commenced. The mixture was kept for 2 hours at this temperature, and the resulting yellow syrup dissolved in dilute hydrochloric acid. Addition of sodium hydroxide gave the *3′-methyl-4-phenylpyridinoglyoxaline*, which formed, from light petroleum (b. p. 100—120°), elongated prisms (4·8 g.), m. p. 108—110° (Found: C, 80·4; H, 5·9; N, 13·5. $C_{14}H_{12}N_2$ requires C, 80·7; H, 5·8; N, 13·5%). It forms a *picrate* (yellow needles from ethanol–acetic acid), m. p. 240—241° (decomp.) after sintering at 210° (Found: N, 15·9. $C_{20}H_{15}O_7N_5$ requires N, 16·0%).

2-2′-*Pyridylamino*cyclo*hexanone*.—2-Chloro*cyclo*hexanone (5 g.), 2-aminopyridine (4·5 g.), and sodium carbonate (3 g.) were boiled in ethanol for 3 hours. Trituration followed by evaporation of the solvent gave 2-2′-*pyridylamino*cyclo*hexanone* (2·1 g.) (needles from ethanol), m. p. 147—149° (Found: C, 69·1; H, 7·5; N, 15·2. $C_{11}H_{14}ON_2$ requires C, 69·5; H, 7·4; N, 14·7%). 2 : 4-Dinitrophenylhydrazine and sulphuric acid gave by Brady's method the impure 2 : 4-dinitrophenylhydrazone sulphate (orange needles from ethanol–acetic acid), m. p. 164—165° (decomp.) (Found: N, 16·4; S, 6·2. $C_{17}H_{20}O_8N_6S$ requires N, 18·0; S, 6·8%). The pyridylamino*cyclo*hexanone (0·7 g.), sodium hydroxide (3 g.), and water (5 ml.) were boiled, and enough ethanol added to give a homogeneous solution. Boiling was continued for 9 hours and the solution was diluted with water. Extraction with ether and subsequent evaporation gave a brown oil which solidified on trituration with ethanol to give 2-2′-*pyridylamino*cyclo*hexanol* (rhombic plates from ethanol), m. p. 159—160° (Found: C, 68·3; H, 8·4; N, 14·4. $C_{11}H_{16}ON_2$ requires C, 68·7; H, 8·4; N, 14·6%). The same product was obtained by

reduction with aluminium *iso*propoxide in the usual way ("Organic Reactions," ed. Roger Adams, Vol. II, p. 197) except that methanol instead of ethanol was used in the Hahn partial condenser. Boiling of the pyridylamino*cyclo*hexanone (2·3 g.) in water (25 ml.) and concentrated hydrochloric acid (35 ml.) with amalgamated zinc (*ca.* 25 ml.) for 10 hours, with concentrated hydrochloric acid (25 ml.) added every 3 hours, gave 2-aminopyridine (1·5 g.), identified as the picrate, m. p. 218—221° (decomp.), and as the dibenzoyl derivative (needles from ethanol), m. p. 167—169° (Found: C, 75·5; H, 4·7; N, 9·8. Calc. for $C_{19}H_{14}O_2N_2$: C, 75·5; H, 4·7; N, 9·3%), giving no m. p. depression when mixed with an authentic sample of 2-dibenzoylaminopyridine.

2-Pyridylamino*cyclo*hexanone (2·8 g.), sodium hydroxide (2 g.), and 90% hydrazine hydrate (5 ml.) were boiled in trimethylene glycol (40 ml.) for 1 hour, and the excess of hydrazine and water were distilled off until the temperature of the boiling liquid reached 200°. The solution was then boiled for 4 hours and diluted with water, whereupon 2-2′-pyridylamino*cyclo*hexane crystallised out as a mass of colourless plates (0·9 g.), m. p. 125—126° (lit., 123—124°). It gave no m. p. depression when mixed with the compound prepared as follows (cf. Bergstrom, *J. Org. Chem.*, 1946, **11**, 244). 2-Bromopyridine (5 g.) and *cyclo*hexylamine (9·5 g.) were boiled for 6 hours, and the cooled mixture dissolved in pyridine. Powdered sodium hydroxide was added, the pyridine and excess of *cyclo*hexylamine removed by evaporation, and the residue distilled. 2-Bromopyridine (b. p. 105—110°/44 mm.) distilled over and 2-2′-pyridylamino*cyclo*hexane separated on the side-arm of the distillation flask; the latter formed needles (from light petroleum), m. p. 125—126° (Found: C, 74·8; H, 8·8. Calc. for $C_{11}H_{16}N_2$: C, 75·0; H, 9·1%). It gave a *benzoyl* derivative (needles from ethanol), m. p. 129—130° (Found: C, 77·0; H, 7·1; N, 9·8. $C_{18}H_{20}ON_2$ requires C, 77·1; H, 7·2; N, 9·9%), and a *picrate* (yellow needles from ethanol–acetic acid), m. p. 185—187° (Found: N, 17·2. $C_{17}H_{19}O_7N_5$ requires N, 17·3%).

2-2′-Pyridylamino*cyclo*hexanone (0·9 g.) was boiled with acetic anhydride (1·6 g.) for 3 hours and then poured into water. Neutralisation with sodium carbonate gave a brown oil which slowly crystallised and on extraction with light petroleum gave 4 : 5 : 6 : 7-tetrahydropyridino(1′ : 2′-1 : 2)-benziminazole as colourless cubes, m. p. and mixed m. p. with a specimen prepared as above 56—58°. A product insoluble in light petroleum was also obtained as yellow prisms (from ethanol), m. p. 158—160° (Found: C, 73·1; H, 6·5; N, 7·3%).

2-2′-Pyridylamino*cyclo*hexanone (2·5 g.) was dissolved in glacial acetic acid and dry hydrogen bromide was led through the cold solution for 1 hour. From the solution an 80% yield of tetrahydropyridino-benziminazole, m. p. 54—58°, was obtained.

Other Cyclisations.—2-Amino-3-methylpyridine (2·5 g.), 2-chloro*cyclo*hexanone (4·6 g.), and sodium carbonate (1·9 g.) were boiled in ethanol (20 ml.) for 3 hours. Chromatographic purification of the product gave unchanged 2-amino-3-methylpyridine, a product, possibly *cyclo*hex-2-enone, and tetrahydro-3′-methylpyridinobenziminazole (46% yield), m. p. 59—61°. Similar experiments with 2-amino-5-bromopyridine (reflux time 12 hours) and 2-amino-3 : 5-dibromopyridine (reflux period 18 hours) yielded the corresponding 5′-bromo- (17% yield), m. p. 147—148°, and 3′ : 5′-dibromo-compounds (30% yield), m. p. 159—160°, respectively.

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UNIVERSITY OF EDINBURGH. [*Received, March 29th*, 1951.]

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534. *Urea Complexes of Some Branched-chain and Cyclic Esters.*

By E. V. TRUTER.

The interaction of forty branched-chain and cyclic esters with urea has been examined and the minimum chain length necessary for complex formation has been determined. It is emphasised that the minimum chain-length is intimately related to the spatial configuration, and the principles for determining whether any particular ester will form a complex have been deduced.

THE original discovery that urea forms complexes with straight-chain organic compounds (Bengen, G.P. Appl. 12438/1940) has been more fully described by Bengen and W. Schlenk (*Experientia*, 1949, **5**, 200) and by W. Schlenk (*Annalen*, 1949, **565**, 204). This work has been extended by Redlich, Gable, Dunlop, and Millar (*J. Amer. Chem. Soc.*, 1950, **72**, 4153), Holman and H. Schlenk (*ibid.*, p. 5001), and Newey, Shokal, Mueller, Bradley, and Fetterly (*Ind. Eng. Chem.*, 1950, **42**, 2538). W. Schlenk (*loc. cit.*) reported that this property was not confined to straight-chain compounds; branched-chain and even cyclic compounds form complexes provided that the straight-chain portion of the molecule is long enough. Only two examples were given,

namely, benzene and 3-methylheptane which do not form addition complexes whereas *n*-octadecylbenzene and 3-methyleicosane do.

Linstead and Whalley (*J.*, 1950, 2987) showed that under suitable conditions certain branched-chain and cyclic esters could be separated from similar straight-chain esters in almost quantitative yield. It was found that methyl 3-methylundecanoate formed a complex while ethyl methylsuccinate, methyl β-methylglutarate, *iso*propyl adipate, methyl ββ′-dimethylsuberate, methyl salicylate, and ethyl benzoate did not. They pointed out that branched-chain esters failed to give stable complexes unless there was a long straight-chain sector in the molecule. Zimmerschied, Dinerstein, Weitkamp, and Marschner (*Ind. Eng. Chem.*, 1950, **42**, 1300) confirmed that the linearity of the alkyl chain is a predominant factor, derivatives of slightly branched alkanes being able to form adducts if the straight portion is long enough. It appeared to them that two methyl groups or an ethyl or a phenyl side-group could prevent complex formation altogether. They found that the *iso*propyl esters of oleic, eicos-11-enoic * and docos-13-enoic acids, methyl 12-methylmyristate,* and 12-methyltetradecyl acetate formed complexes, whereas 1-methylheptyl acetate, *iso*propyl 12-methylmyristate, methyl 9-phenylstearate, and 2-ethylhexyl myristate did not.

In order to permit this technique to be used for the separation of material (*e.g.*, wool wax) containing straight-chain, branched-chain, and cyclic molecules, a more systematic investigation into the minimum length of the anchor necessary to enable a particular group to be held in a complex has been carried out. Contrary to the statement by Zimmerschied *et al.* compounds containing an ethyl side chain or a phenyl group can be made to give complexes provided that certain spatial requirements are fulfilled.

The importance of the configuration is well illustrated by the amyl alcohol series where the minimum carbon chain-length of the acid necessary to enable the ester to form a complex ranges from two atoms to ten. The carbonyl group, though somewhat larger than a methylene group, does not hinder complex formation. The introduction of a side-chain methyl group leads to a slight increase in the cross-sectional area if it is on the same side of the molecule as the carbonyl oxygen atom, and to a larger increase if it is on the opposite side.

Ester derived from	Minimum carbon chain length of acid †	Total straight chain
n-Amyl alcohol	2 (acetate)	8
3-Methylbutanol	6 (hexanoate)	11
2-Methylbutanol	10 (decanoate)	15

† Including the oxygen atom of the C–O–C grouping.

Ester derived from	Minimum chain length from nucleus.†	Ester derived from	Minimum chain-length from nucleus.
Phenol	9 (octanoate)	Phenylacetic acid	13 (decanol)
Quinol	2 × 5 (dibutyrate)	*cyclo*Hexanol	13 (dodecanoate
Benzyl alcohol	12 (decanoate)	Benzoic acid	(20 not sufficient)

† Including the oxygen atom of the C–O–C grouping.

In the series containing an aromatic nucleus, benzoic esters provide an example of the cross-sectional area becoming too large for the space available.

Comparison of the behaviour of derivatives of quinol and phenol, *iso*amyl alcohol and 1-methylheptanol, and 2-ethylbutanol and 2-ethylhexanol, shows that the effectiveness of the straight portion is not appreciably diminished if it is in two separate parts.

It may appear at first sight that isomers could be separated by using suitable derivatives. However, a compound that does not form a complex by itself may give a mixed complex in the presence of one that does, *e.g.*, 3-methylheptane gives a mixed complex with *n*-octane (W. Schlenk). Linstead and Whalley have shown that the converse may occur; a compound that normally forms a complex may be prevented from doing so by the presence of one that does not. In either case only a partial separation will be achieved. This is demonstrated further by the amyl alcohol series. By itself 3-methylbutyl hexanoate forms an addition product, but not in the presence of 2-methylbutyl hexanoate. Conversely, 2-methylbutyl octanoate, normally unable to form an adduct, gives a mixed addition product with 3-methylbutyl octanoate. In the latter case, however, the 3-isomer is preferentially removed by urea and, for a countercurrent process, the enrichment factor is large enough to allow the separation of optically active amyl alcohol from a mixture containing *iso*amyl alcohol.

Members of a homologous series may be partially separated by treating the mixture with less than the equivalent amount of urea. For example, a mixture containing equal quantities of

* Geneva nomenclature (CO_2H = 1) is used throughout the paper.

octadecyl dodecanoate and octadecyl palmitate can be separated into four fractions of which the one with the highest affinity for urea contains 75% of palmitate and that with the lowest affinity only 14%. Qualitative results are given in the following table.

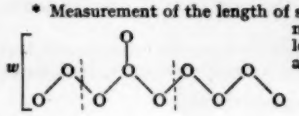
Complex-forming.	Unable to form complexes.
n-Octadecyl acetate	*n*-Octadecyl benzoate
n-Octadecyl dodecanoate	
n-Octadecyl palmitate	
Dioctadecyl sebacate	Cholesteryl acetate
	Cholesteryl octanoate
	Cholesteryl palmitate
Phenyl dodecanoate	
Phenyl octanoate (weak)	Phenyl hexanoate
Benzyl dodecanoate	
Benzyl decanoate	Benzyl octanoate
*cyclo*Hexyl dodecanoate	*cyclo*Hexyl octanoate
	*cyclo*Hexyl decanoate
Dodecyl phenylacetate	
Decyl phenylacetate	
Oleyl alcohol	
Dibromo-oleyl alcohol	
	Geraniol
Geranyl dodecanoate	Geranyl octanoate
Citronellyl octanoate	Citronellol
	Citronellyl butyrate
3-Methylbutyl octanoate	3-Methylbutanol
3-Methylbutyl hexanoate	3-Methylbutyl propionate
2-Methylbutyl dodecanoate	2-Methylbutyl propionate
2-Methylbutyl decanoate	2-Methylbutyl octanoate
2-Ethylbutyl dodecanoate	2-Ethylbutanol
2-Ethylbutyl decanoate	2-Ethylbutyl octanoate
2-Ethylhexyl dodecanoate	2-Ethylhexyl hexanoate
2-Ethylhexyl octanoate	
	1-Methylheptyl acetate
1-Methylheptyl butyrate	1-Methylheptyl propionate
β-Naphthyl palmitate	3 : 9-Diethyltridecan-6-ol
Quinol dihexanoate	3 : 9-Diethyl-6-tridecyl propionate
Quinol dibutyrate	3 : 9-Diethyl-6-tridecyl nonanoate

W. Schlenk was unable to obtain an addition product from a hydrocarbon $C_{88}H_{118}$, obtained from montanic acid, and this led him to suppose that there might be an upper limit to the length of the chain that can give an adduct. However, if there is an upper limit it seems unlikely that it will be as low as this because octadecyl palmitate (chain length 35 atoms) and dioctadecyl sebacate (chain length 48 atoms) give complexes without difficulty. Moreover, Zimmerschied *et al.* had no difficulty in preparing an adduct from a hydrocarbon fraction containing an average of fifty carbon atoms per molecule.

The behaviour of esters may be summarised as follows: (i) All normal esters containing chains of 9—48 atoms give addition products with urea. (ii) For each side-chain methyl (or ethyl) group on the same side of the saturated main chain as the carbonyl oxygen atom a straight chain of at least 8 atoms * is required to enable the substance to form a complex. If substitution occurs on opposite sides of the molecule, at least 11 atoms * per side-group are required. (iii) A main-chain phenyl group on the same side as the carbonyl-oxygen atom required a straight chain of 9 atoms, whereas the slightly larger *cyclo*hexyl group requires 13 atoms, to enable it to form a complex. (iv) Benzoates do not form complexes. (v) Two short chains are as effective as one long one.

There is only one exception to these rules amongst the 16 non-linear esters examined by previous authors: from 2-ethylhexyl myristate Zimmerschied *et al.* were unable to obtain an adduct. Tested by the method described below, however, lower homologues (octanoate and dodecanoate) gave complexes without difficulty.

* Measurement of the length of straight-chain does not include the atoms at the widest point of the molecule. Thus, the width is *w* as shown annexed, and the chain-length 6 (not 9). This allows direct comparison of aromatic with aliphatic esters.



Experimental.

Test for Complex Formation.—Urea (3 g.) was moistened with methanol (0·5 ml.), and 50 ml. of a 1% solution of the ester in benzene were added. Next morning the solids were filtered off, washed with benzene (3 × 20 ml.), and dried at room temperature. A positive result for complex formation was indicated by the appearance of an oil or a finely divided solid when the complex was dissolved in water.

Quantitative Separation of Esters.—The method used was very similar to that described by Linstead and Whalley (*loc. cit.*). A mixture of the two esters (2 g. each) was dissolved in light petroleum (100 ml., b. p. 40—60°) and added to urea (10 g.) moistened with methanol (2 ml.). Next morning the solids were filtered off, washed with light petroleum (b. p. 40—60°; 3 × 30 ml.), decomposed with water (100 ml.), and extracted with ether (3 × 100 ml.). The ethereal solution was washed with water (3 × 100 ml.) and dried (Na_2SO_4), and the complex-forming component obtained by distillation of the solvent. The filtrate was washed with water, dried, and evaporated to give the other compound. The following results were obtained (data are for crude materials as obtained from the separations):

(i) *n*-Octadecyl acetate (m. p. 32°; negative Liebermann–Burchardt test) was separated from cholesteryl acetate (m. p. 111°) in 94 and 95% yield respectively; starting materials had m. p. 32° and 113°.

(ii) Octadecyl palmitate (m. p. 57°; negative Liebermann–Burchardt test) was separated from cholesteryl octanoate, m. p. 102°, in 94 and 98% yield respectively. Starting materials had m. p. 57° and 104°.

(iii) Octadecyl palmitate (m. p. 57°; trace of colour in the Liebermann–Burchardt test) was separated from cholesteryl palmitate (m. p. 78°) in 97 and 94% yield respectively. Starting materials had m. p. 57° and 78°.

(iv) Octadecyl acetate (m. p. 31°) was separated from lanosteryl acetate to give 29% of octadecyl acetate and a mixture in the uncombined fraction.

Separation of Octadecyl Dodecanoate from Octadecyl Palmitate.—Equal quantities (2 g.) of the two esters, dissolved in light petroleum (100 ml.), were added to urea (6 g.) moistened with methanol (1 ml.). Next morning the two fractions were isolated as described above. Each fraction was again treated with urea (3 g.) and methanol (0·5 ml.). There were obtained fractions: (i), m. p. 52—55·5°, sap. val. 114 (octadecyl palmitate has m. p. 57° and sap. val. 110·5); and (iv), m. p. 40—43°, sap. val. 122·5 (octadecyl dodecanoate has m. p. 42° and sap. val. 124·5). The saponification values indicate that fraction (i) contained about 75% of palmitate, and fraction (iv) about 86% of dodecanoate.

Separation of the Optically Active Constituent from Commercial Amyl Alcohol.—The alcohol mixture (as used for testing of milk) was converted into the octanoates by treatment with octanoyl chloride, $[\alpha]_D^{18}$ +0·54°. The esters (40 g.), dissolved in light petroleum (200 ml.), were treated overnight with urea (100 g.) and methanol (30 ml.) and worked up in the usual manner. Fraction (1) (13·5 g.) did not combine with urea and had $[\alpha]_D^{18}$ +0·71°; fraction (2) had $[\alpha]_D^{18}$ +0·35°. Fraction (1) was again treated with urea (35 g.) and methanol (10 ml.) to give fraction (3) (uncombined; 6·5 g.), $[\alpha]_D^{18}$ +1·06°. Fraction (3) was treated with urea (6 g.) and methanol (1 ml.), to give fraction (4) (uncombined; 4·5 g.), $[\alpha]_D^{18}$ +1·19°.

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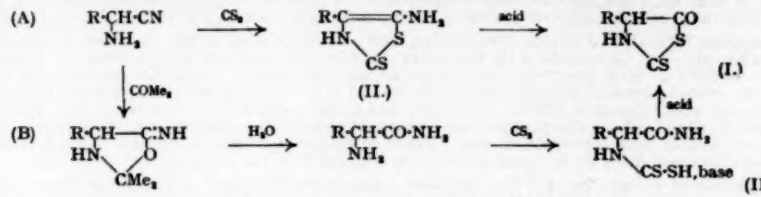
535. *Peptide Synthesis from Heterocyclic Intermediates. Part I. 2-Thio-5-thiazolidone Derivatives of Valine, Leucine, Norleucine, Methionine, L-Tyrosine, Glutamine, α-Aminoisobutyric Acid, and Aminomalonamide.*

By A. C. Davis and A. L. Levy.

The thiothiazolidones mentioned in the title are made by cyclisation of the dithiocarbamates of the appropriate α-amino-amides with aqueous mineral acid; the mechanism of this reaction is discussed. The valine derivative is better prepared by hydrolysis of the corresponding 5-amino-2-mercaptothiazole. Representative members of this thiothiazolidone series have been found to racemise with great ease.

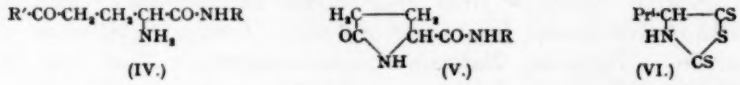
Cook, Heilbron, and Levy (*J.*, 1948, 201) prepared a 2-thio-5-thiazolidone (I; R = H) by routes (A) and (B), of which the latter, though longer, was more convenient preparatively. Billimoria and Cook (*J.*, 1949, 2323) introduced a third general route (C), namely, reduction with zinc and hot acetic acid of the corresponding 4-alkylidene- or 4-arylidene-thiazolones,

which were readily obtained by condensation of (I; R = H) with aldehydes or ketones. By this method they prepared 2-thio-5-thiazolidones (I; R = CH_2Ph, *p*-$OH{\cdot}C_6H_4{\cdot}CH_2$, *cyclo*hexyl, and Et) related to phenylalanine, tyrosine, *C-cyclo*hexylglycine, and α-aminobutyric



acid. The corresponding derivative of *C*-phenylglycine (I; R = Ph) was obtained by route (B). Cook and Levy (*J.*, 1950, 642) prepared the alanine thiothiazolidone (I; R = Me) by route (B), though route (A) was not successful in this case. A number of related *N*-methyl-2-thio-5-thiazolidones have also been made by appropriate modifications of all three routes (Fourneau and Vila, *Bull. Soc. chim.*, 1911, **9**, 985; Carrington, *J.*, 1948, 1619; Cook and Cox, *J.*, 1949, 2342). The finding that 2-thio-5-thiazolidone (I; R = H) could be used to effect a ready synthesis of glycyl-polypeptides (Cook and Levy, *J.*, 1950, 646), and that route (B) presented a method for the stepwise degradation of peptides (Levy, *J.*, 1950, 404), made it desirable to extend the available range of these compounds, and the present communication records the synthesis of eight new 2-thio-5-thiazolidones, related to both natural and artificial α-amino-acids. These compounds were briefly reported earlier (Levy, *loc. cit.*).

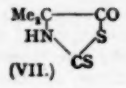
L-Tyrosine, L-leucine, and DL-methionine were converted by known methods into their methyl esters, and thence by reaction with ammonia into their amides. These were condensed with carbon disulphide, and the resulting dithiocarbamates (isolated in the case of L-tyrosine as a crystalline triethylamine salt) acidified with aqueous hydrochloric acid to yield 4-*p*-hydroxybenzyl- (I; R = *p*-$OH{\cdot}C_6H_4{\cdot}CH_2$), 4-*iso*butyl- (I; R = Bu^i), and 4-2′-methylthioethyl-2-thio-5-thiazolidine (I; R = $MeS{\cdot}CH_2{\cdot}CH_2$). In the case of tyrosine, the thiothiazolidone was optically active, but racemised during 2—3 hours in methanol solution. However, in the presence of 0·1 equivalent of hydrogen chloride, or in ethanol solution, it was considerably more stable, and little or no racemisation was apparent in ethyl acetate. In the presence of bases, optical rotation was rapidly lost, and although tyrosylglycylglycine ethyl ester hydrochloride was secured in 78% yield from glycylglycine ethyl ester by Cook and Levy's procedure (*loc. cit.*), it was found to be optically inactive. When freshly prepared, the crude leucine thiothiazolidone was also optically active, but it appeared to racemise with great ease, and only inactive material was recovered after crystallisation. These results are very similar to those obtained with simple oxazolones (" The Chemistry of Penicillin," Princeton Univ. Press, 1949, p. 742), though it is possible that by careful control of pH peptide syntheses may be effected without prohibitive racemisation.

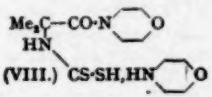


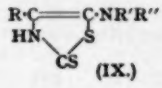
Ammonolysis of dimethyl or diethyl glutamate produces almost exclusively pyrrolidone-5-carboxyamide (V; R = H), so alternative methods to glutamic amides (IV) were used. The L-α-anilide (IV; R = Ph, R′ = OH) was prepared enzymically by condensing *N*-carbobenzyloxy-L-glutamic acid with aniline in the presence of papain, and removing the protecting group by hydrogenation (Behrens and Bergmann, *J. Biol. Chem.*, 1939, **129**, 594). It was shaken with carbon disulphide and sodium carbonate solution but acidification did not yield the required thiothiazolidone. *N*-Carbobenzyloxy-L-glutamic acid α-anilide was therefore esterified with methanolic hydrogen chloride, but hydrogenation of the resulting γ-methyl *N*-carbobenzyloxy-L-glutamate α-anilide yielded only pyrrolidone-5-carboxyanilide (IV; R = Ph), despite the mild reaction conditions employed. Difficulties were also experienced in using L-*iso*glutamine (IV; R = H, R′ = OH), and the final successful approach utilised the hitherto unknown L-glutamamide (IV; R = H, R′ = NH_2), which was, however, not isolated. Catalytic hydrogenation of *N*-carbobenzyloxy-L-glutamamide (Fruton, *J. Biol. Chem.*,

1946, **165**, 333) was rendered difficult by its insolubility in common solvents, but debenzylation with sodium in liquid ammonia (Loring and Du Vigneaud, *J. Biol. Chem.*, 1935, **111**, 385) yielded crude glutamamide, which on treatment with carbon disulphide in methanol, followed by acidification, gave 4-2′-carbamylethyl-2-thio-5-thiazolidone (I; R = NH_2·CO·CH_2·CH_2) in poor yield. It proved to be optically inactive.

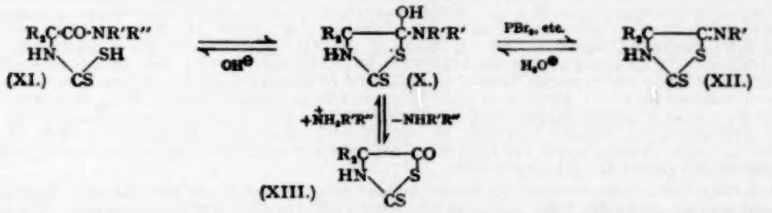
In the valine series, an entirely synthetic approach from *iso*butaldehyde was adopted; this was converted successively into the cyanohydrin and aminonitrile, which by route (B) was transformed through four stages into the required 4-*iso*propyl-2-thio-5-thiazolidone (I; R = Pr^i). The thiazolidone proved to be remarkably stable to acids, so that in this case route (A) became the procedure of choice, 5-amino-2-mercapto-4-*iso*propylthiazole (II; R = Pr^i) being deaminated to (I; R = Pr^i) in 66% yield when warmed with 3N-hydrochloric acid. In the course of attempts to obtain the sulphur analogue (VI) of (I) by treating (II) with hydrogen sulphide, 5-*iso*propyl-2 : 4-dithiohydantoin was obtained, and identified with the product of alkaline rearrangement of (II; R = Pr^i) (cf. Cook, Heilbron, and Levy, *J.*, 1947, 1598). The 2-thio-5-thiazolidones of glycine, alanine, methionine, and valine form a series showing increasing stability to hydrolysis. Thus (I; R = H) is hydrolysed rapidly by water at 70° (I; R = Me) in 1—2 minutes at 100°, and (I; R = MeS·CH_2·CH_2) in about 5 minutes at 100°; (I; R = Pr^i) is stable for a prolonged period to boiling water, and refluxing for about 5 minutes with concentrated hydrochloric acid is needed to cause complete breakdown.


(VII.)


(VIII.)


(IX.)

α-Amino*iso*butyronitrile did not undergo satisfactory condensation with acetone in the presence of sodium methoxide, but the amide sulphate was readily secured by mixing the aminonitrile with concentrated sulphuric acid, and pouring the whole into ethanol (cf. Cook and Cox, *J.*, 1949, 2334). The remaining stages of route (B) then proceeded normally, giving 4 : 4-dimethyl-2-thio-5-thiazolidone (VII) in 86% yield. With morpholine in acetone, this afforded morpholinium 2-formomorpholidyl-2-propyldithiocarbamate (VIII) in the usual way, morpholinium dithiocarbamate being obtained under slightly more vigorous conditions. It had hitherto been supposed (A. L. Levy, Ph.D. Thesis, London, 1947) that the mechanism of the remarkable fission of the peptide linkage by NH·CS·SH in compounds such as (III) involved a momentary cyclisation to the 5-amino- or 5-imino-thiazole derivatives (IX) or (XII), followed at once by hydrolysis to the 2-thio-5-thiazolidones. This suggestion arose from the evidence presented by Cook, Heilbron, and Levy (*loc. cit.*) that the *S*-benzyl ester of (III; R = H) was quantitatively transformed into the *S*-benzyl derivative of (II; R = H) with phosphorus tribromide, and that (II; R = H) was hydrolysed by acids to (I; R = H). It also seemed to account for the fact that (I; R = H) was obtained from (III) under hydrolysing conditions, but not by the action of anhydrous acids. It was of particular interest, therefore, to find that the morpholine derivative (VIII), in which such a mechanism could not operate, was readily recyclised to (VII) in 93% yield with dilute mineral acid.

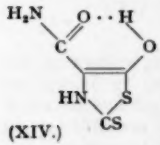

(XI.) (X.) (XII.) (XIII.)

It would appear, then, that fission of the peptide linkage must take place *via* the ortho-acid derivative (X), which can be represented as the common intermediate in all the above-mentioned transformations: (*a*) α-amino-amide dithiocarbamate ⟶ 2-thio-5-thiazolidone; (XI) ⟶ (X) ⟶ (XIII) under acid conditions; (*b*) 2-thio-5-thiazolidone + amine ⟶ α-amino-amide dithiocarbamate; (XIII) ⟶ (X) ⟶ (XI) under basic conditions; (*c*) α-

thioacylamino-amide ⟶ 5-aminothiazole; (XI) ⟶ (X) ⟶ (XII) under dehydrating conditions; (*d*) 5-aminothiazole ⟶ thiazol-5-one; (XII) ⟶ (X) ⟶ (XIII) with aqueous acid. This series is analogous to the "ester" or "amide" fission of imino-ethers with acids or bases. It is interesting that Hanby, Whaley, and Watson (*Proc. Roy. Soc.*, 1949, *A*, **199**, 499) have suggested the intervention of an intermediate similar to (X), to account for the kinetics of polymerisation of *N*-carboxysarcosine anhydride. The driving force of the reaction (X) ⟶ (XIII) must arise in part from the aromaticity of the thiazolone ring, since the dithiocarbamate of β-alanine amide did not cyclise to a thiazine derivative on acidification.

For preparations in the norleucine series, *n*-hexanoic acid was a convenient starting material. Bromination by the Hell–Volhard–Zelinsky method yielded the known 2-bromo-*n*-hexanoyl bromide, which gave DL-norleucine amide directly by the action of methanolic ammonia. This afforded a crystalline dithiocarbamate salt with carbon disulphide, which cyclised to 4-*n*-butyl-2-thio-5-thiazolidone (I; R = $Me\cdot[CH_2]_3$) in 78% overall yield on acidification in water.

4-Carbamyl-2-thio-5-thiazolidone (I; R = $CO\cdot NH_2$) was obtained by route (B) in 90% yield from aminomalonamide, either by shaking the amide in aqueous sodium carbonate with carbon disulphide, or, better, by conversion into the crystalline dithiocarbamate by heating a suspension of the highly insoluble amide in pyridine with carbon disulphide under reflux. It was rather less stable than the other thiothiazolidones which have been made, decomposing gradually on storage and losing carbon disulphide and carbon dioxide with effervescence when boiled with water. An interesting possibility arises with (I; R = $CO\cdot NH_2$), in that the products of reaction with bases (III; R = $CO\cdot NR'R''$) can cyclise in two ways with acids, eliminating either ammonia or the base, and it was hoped that such experiments would indicate the relative efficiency of different amines in promoting ring closure. In fact, (I; R = $CO\cdot NH_2$) yielded crystalline derivatives on treatment with aniline, benzylamine, and morpholine, and all of these gave back (I; R = $CO\cdot NH_2$) on acidification. Analysis showed, however, that the morpholine adduct contained only one molecule of the base, and was presumably, therefore, a morpholine salt. A similar result has been noted in the case of 4-carbethoxy-2-phenyl-5-oxazolone, which gives a salt with benzylamine, converted into the normal benzylamide by heating it in xylene (*op. cit.*, p. 787). This behaviour can probably be ascribed to hydrogen-bond formation, as shown in (XIV), a view which receives strong support from the fact that (I; R = $CO\cdot NH_2$), and its morpholine salt, give an intense indigo-blue colour with ferric chloride. A similar tendency may well account for the rather exceptional properties of the 5-amino-4-carbethoxyazoles, encountered by Cook, Heilbron, *et al.* (*locc. cit.*).



(XIV.)

All the thiothiazolidones here mentioned have been characterised as anhydrides by reaction with hydroxylamine, the resulting hydroxamic acid giving an intense brown colour with ferric chloride (cf. *op. cit.*, p. 1029).

Experimental.

L-4-p-*Hydroxybenzyl-2-thio-5-thiazolidone*. (This and the succeeding preparation were carried out by R. Sayers and L. Steensgaard.)—L-Tyrosine amide (Könige and Mylo, *Ber.*, 1908, **41**, 4441) (1·0 g.) in ethanol (16 c.c.) was treated with carbon disulphide (2·0 c.c.) and triethylamine (2·0 c.c.), and the resulting crystalline triethylamine salt of L-tyrosine amide dithiocarbamate (1·9 g., 96%), $[\alpha]_D^{25}$ +43·0° → +0·4° (*c*, 3·4 in water), was filtered off after 2 hours at room temperature. The salt in water (40 c.c.) was treated with 4N-hydrochloric acid; on seeding, almost colourless L-4-p-*hydroxybenzyl-2-thio-5-thiazolidone* (1·1 g., 93%) separated; recrystallised from ethyl acetate–light petroleum (b. p. 60—80°), it had m. p. 154° (Found: C, 50·5; H, 3·85; N, 5·9; S, 26·6. $C_{10}H_9O_2NS_2$ requires C, 50·2; H, 3·8; N, 5·85; S, 26·8%), $[\alpha]_D^{25}$ +174° (*c*, 2·4 in methanol). The thiazolidone (0·72 g.) and glycylglycine ethyl ester hydrochloride (0·59 g.) were shaken with ethanol (10 c.c.) containing triethylamine (0·83 c.c.), and after 10 minutes 1 equivs. of hydrogen chloride in ethanol, followed by a little ether, were added to precipitate the peptide (0·85 g., 78%), m. p. 209—210° (decomp.). DL-*Tyrosylglycylglycine ethyl ester hydrochloride*, recrystallised from methanol–ether, had m. p. 212° (decomp.) (Found: C, 49·65; H, 5·6; N, 11·2. $C_{15}H_{22}O_5N_3Cl$ requires C, 50·0; H, 6·1; N, 11·7%); it gave a single spot (R_F 0·76) on a butanol–acetic acid paper chromatogram and gave rise to tyrosine and glycine (1 : 2) on hydrolysis.

4-iso*Butyl-2-thio-5-thiazolidone*.—L-Leucine amide, prepared by Yang and Rising's method (*J. Amer. Chem. Soc.*, 1931, **53**, 3183), had m. p. 101—102°, $[\alpha]_D^{25}$ −34·8° (*c*, 1·27 in chloroform). The amide (0·25 g.) in ethanol (4 c.c.) was kept with triethylamine (0·5 c.c.) and carbon disulphide (0·5 c.c.) for 1 hour at room temperature, and poured into 6N-hydrochloric acid (20 c.c.). The thiazolidone was extracted with chloroform (4 × 10 c.c.), and the lævorotatory extract (α −1·07°, *l* = 1) was dried and evaporated in a vacuum to give a very pale yellow solid, m. p. 89—90°. 4-iso*Butyl-2-thio-5-thiazolidone* crystallised from light petroleum (b. p. 60—80°) in almost colourless needles, m. p. 94° (Found: C, 44·6; H, 5·9. $C_7H_{11}ONS_2$ requires C, 44·4; H, 5·9%). The colour could be completely removed by adsorption on alumina from benzene solution. The recrystallised material was optically inactive.

4-2′-*Methylthioethyl*-2-*thio*-5-*thiazolidone*.—DL-Methionine amide (1·68 g.) (Jones, *J. Amer. Chem. Soc.*, 1949, **71**, 79) in ethanol (20 c.c.) at 0° was treated with carbon disulphide (1 c.c.). After 1 hour at 0°, the solution was evaporated in a vacuum at 25°. Addition of ice-cold concentrated hydrochloric acid (10 c.c.) to a solution of the residue in water (7·5 c.c.) at 0° gave an oil which soon crystallised (0·92 g., 95%). After recrystallisation from *cyclo*hexane the 2-*thio*-5-*thiazolidone* formed colourless needles, m. p. 97° (Found: N, 6·7. $C_6H_9ONS_2$ requires N, 6·8%).

4-2′-*Carbamylethyl*-2-*thio*-5-*thiazolidone and Related Compounds*.—L-Glutamic acid α-anilide (Behrens and Bergmann, *loc. cit.*) (300 mg.) was shaken overnight in 2N-aqueous sodium carbonate (2 c.c.) with carbon disulphide (0·5 c.c.). Extraction with ethyl acetate after acidification with mineral acid yielded only an unidentified gummy solid (20 mg.).

N-Carbobenzyloxy-L-glutamic acid α-anilide (Behrens and Bergmann, *loc. cit.*) (5 g.) in dry methanol (250 c.c.) at room temperature was treated with saturated methanolic hydrogen chloride (2·5 c.c.). After 12 hours the crystalline product was collected, and a further quantity obtained by concentration of the filtrate in a vacuum; the combined products were dissolved in chloroform and precipitated with light petroleum to yield *γ-methyl* N-*carbobenzyloxy*-L-*glutamate* α-*anilide* (4·9 g., 94%), which crystallised from ethanol in colourless needles, m. p. 152° (Found: C, 65·1; H, 6·05; N, 7·6. $C_{20}H_{22}O_5N_2$ requires C, 64·85; H, 6·0; N, 7·6%). On hydrogenation over 5% palladium–charcoal in ethyl acetate containing 1 equivalent of acetic acid, the substance (3·6 g.) took up the theoretical volume of hydrogen; evaporation at a low temperature in a vacuum gave an oil which did not react with carbon disulphide in ethanol, but on being kept overnight in that solvent (10 c.c.) deposited crystalline pyrrolid-2-one-5-carboxyanilide (1 g.), m. p. 186—187° (Found: C, 64·7; H, 5·9; N, 13·9. Calc. for $C_{11}H_{12}O_3N_2$: C, 64·8; H, 5·9; N, 13·7%).

N-Carbobenzyloxy-L-glutamamide was obtained by Fruton (*loc. cit.*) by esterification of L-glutamic acid and then carbobenzyloxylation, to give the oily dimethyl *N*-carbobenzyloxy-L-glutamate, which was converted directly into the amide. In the present instance, *N*-carbobenzyloxy-L-glutamic acid (15 g.) in dry methanol (500 c.c.) was treated with hydrogen chloride in a rapid stream for 1 hour, with ice-cooling. After 24 hours at room temperature, the solution was evaporated in a vacuum to a syrup, which was taken up in chloroform (80 c.c.), washed with dilute hydrochloric acid, and with aqueous sodium hydrogen carbonate until neutral, dried, and re-evaporated to give the dimethyl ester as an oil (14 g.). On treatment with methanolic ammonia according to Fruton (*loc. cit.*), the diamide (10·5 g.; 67% from *N*-carbobenzyloxy-L-glutamic acid) separated gradually during 3 days. The substance did not absorb hydrogen in suspension in aqueous acetic acid in presence of a palladium catalyst, but a solution of the amide (4 g.) in liquid ammonia (400 c.c.) took up *ca.* 4 atoms (1·24 g.) of sodium before appearance of a permanent blue colour. Acidification with ammonium iodide (7·8 g.) and evaporation gave a hygroscopic solid which could not be cleanly separated to give the desired L-glutamamide; it was therefore extracted twice with cold ethanol (30 c.c., 20 c.c.) to remove sodium iodide, and the hygroscopic residue dissolved in the minimum of methanol and kept overnight at 0° with excess of carbon disulphide. Evaporation in a vacuum gave a solid residue, which was treated, in water (5 c.c.) at 0°, with concentrated hydrochloric acid (5 c.c.). Extraction with ethyl acetate (3 × 15 c.c.) and evaporation of the dried extracts in a vacuum gave a semi-solid residue; on precipitation from ethyl acetate with light petroleum, the 4-2′-*carbamylethyl*-2-*thio*-5-*thiazolidone* (25 mg.) formed lightly-coloured needles, m. p. 143—145°. The compound imparted blue or green colours to organic solvents, from which it could not be satisfactorily crystallised; on a micro-scale it was observed to crystallise well from water, but crystallisation of a larger sample failed owing to gross decomposition (Found, on unrecrystallised material: N, 14·3. $C_6H_9O_2N_3S_2$ requires N, 13·7%). The thiothiazolidone was optically inactive; kept in acetone with morpholine overnight, it yielded colourless needles of the morpholine derivative, m. p. 224°.

L-Glutamic acid (10 g.) was converted into the dimethyl ester hydrochloride by Fruton's method (*loc. cit.*); the free base was liberated by neutralisation in methanol with sodium methoxide, and added to saturated methanolic ammonia (400 c.c.) at 0°. After 7 days at room temperature, 2N-ethanolic hydrogen chloride (30 c.c.) was added, and excess of ammonia and methanol removed in a vacuum. The residue was treated with methanol (50 c.c.) and carbon disulphide (10 c.c.), neutralised with sodium methoxide, and kept overnight at 0°. After evaporation in a vacuum, the semi-solid residue, in the minimum of water, was acidified with concentrated hydrochloric acid. By extraction with ethyl acetate as in the preceding experiment, the product was obtained as a crystalline solid (0·25 g.); recrystallisation from acetic acid gave very pale yellow needles, m. p. 145—147°, strongly depressed by admixture with the above glutamine 2-thiothiazolidone. Analysis confirmed that the compounds were different, suggesting rather the *glutamic acid* derivative (Found: C, 32·0; H, 3·5; N, 6·7. $C_6H_7O_3NS_2$ requires C, 35·1; H, 3·4; N, 6·8. $C_6H_9O_2N_3S_2$ requires N, 13·7%).

4-iso*Propyl*-2-*thio*-5-*thiazolidone*.—The starting material, α-amino*iso*valeronitrile, has been prepared by the action of hydrogen cyanide on the *iso*butaldehyde–ammonia complex (Lipp, *Annalen*, 1880, **205**, 9), but for the present work, it was conveniently prepared by ammonolysis in methanol of the aldehyde cyanohydrin. Since it largely decomposed, without yielding any pure fraction, on distillation in a vacuum, the crude material was employed for the following experiments:

(*a*) α-Amino*iso*valeronitrile (5 g.) and acetone (10 c.c.) (dried over K_2CO_3) were treated with concentrated methanolic sodium methoxide until solid methoxide separated, and left for 7 days at room temperature. The solvent was removed in a vacuum and the residue was treated for 10 minutes with water (10 c.c.) at 60°, and then re-evaporated in a vacuum. The crude valine amide was dissolved in dry ethyl acetate (20 c.c.) and carbon disulphide (5 g.) was added; the semi-solid dithiocarbamate salt separated almost immediately. After 2 hours, all volatile material was removed in a vacuum, and the residue, in water (20 c.c.) at 0°, was acidified with concentrated hydrochloric acid (10 c.c.). Colourless

needles (0·5 g., 11% overall) of 4-iso*propyl*-2-*thio*-5-*thiazolidone*, m. p. 88°, separated at once, and were recrystallised from *cyclo*hexane, the m. p. rising to 101° (Found: C, 41·6; H, 5·4; N, 8·3. $C_6H_9ONS_2$ requires C, 41·1; H, 5·2; N, 8·0%).

(*b*) The amino-nitrile (50 g.) in chloroform (50 c.c.) was treated with carbon disulphide (40 g.), external cooling for 30 minutes being necessary to moderate the reaction. 5-*Amino*-2-*mercapto*-4-iso*propylthiazole* separated in two crops (total yield 48 g., 55%) during 12 hours, in large yellow tablets, m. p. 135—136° (rapid heating), and was recrystallised from chloroform–light petroleum; it had m. p. 141—142° (Found: N, 15·7. $C_6H_{10}N_2S_2$ requires N, 16·1%), and was readily oxidised to a brown pigment in air. The fact that this was the highest yield of the thiazole obtained confirms Lipp's observation (*loc. cit.*) of the spontaneous decomposition of valine nitrile; after 3 weeks at 0°, the latter did not yield any product on treatment with carbon disulphide.

5-Amino-2-mercapto-4-*iso*propylthiazole (10 g.) was powdered finely and shaken with concentrated hydrochloric acid (50 c.c.) until completely replaced by a colourless crystalline precipitate (this deliquescent material, m. p. 143°, contained chlorine, and was probably the thiazole hydrochloride). Water (150 c.c.) was added, and the temperature raised slowly to 60°; after 2 minutes, the crystalline product began to separate, and after 10 minutes, the solution was cooled and kept at 0° for 15 minutes; the thiothiazolidone, which formed large, yellow-tinted needles (6·7 g., 66%), was collected. On recrystallisation from *cyclo*hexane, it formed yellow needles, m. p. 101°, from which the colour could be removed by sublimation at 60° in high vacuum, after which the m. p. was 101—102°.

An attempt to prepare a 2 : 5-dithiothiazolidone, by hydrolysis of the above 5-aminothiazole in cold dilute acid containing hydrogen sulphide, was unsuccessful, the 2-thio-5-thiazolidone being produced. A saturated solution of the thiazole in ethyl acetate was treated with hydrogen sulphide to saturation, and kept for 0° for 4 hours; the yellow crystalline material which was deposited was not a product of reaction with hydrogen sulphide, but proved to be identical with the corresponding dithiohydantoin, prepared as follows (cf. Cook, Heilbron, and Levy, *J.*, 1947, 1598). 5-Amino-2-mercapto-4-*iso*propylthiazole (2 g.) was kept overnight in 10% aqueous sodium hydroxide (20 c.c.) and acidified with hydrochloric acid. The separated 5-iso*propyl*- 2: 4-*dithiohydantoin* (1·6 g., 80%) crystallised from ethyl acetate as yellow prisms, m. p. 232° after darkening and sintering at 200° (Found: C, 42·0; H, 5·9; N, 15·9. $C_6H_{10}N_2S_2$ requires C, 41·4; H, 5·8; N, 16·1%).

5-Amino-2-mercapto-4-*iso*propylthiazole, when heated beyond its m. p., resolidified and remelted at 230°, suggesting that the dithiohydantoin was formed also by thermal rearrangement; in an attempt to isolate it by this method, the thiazole (2 g.) was heated for 5 minutes at 175—180°. Treatment of the crude solid with ethanol left a light yellow powder (0·95 g.), m. p. 240° (decomp.), which recrystallised from acetic acid in yellow plates, m. p. 246° (decomp.). Analytical results showed that the product was stoicheiometrically an addition *compound* of the desired dithiohydantoin and the corresponding 2-thio-5-thiazolidone, but it was not further studied (Found: C, 41·7; H, 5·8; N, 11·7. $C_{12}H_{19}ON_3S_4$ requires C, 41·3; H, 5·45; N, 12·0%).

4 : 4-*Dimethyl*-2-*thio*-5-*thiazolidone*.—α-Amino*iso*butyronitrile (15 g.) was added dropwise to well-stirred sulphuric acid (30 c.c.) containing water (3 c.c.), cooled in ice. The resulting syrup was warmed on the steam-bath to dissolve a small quantity of crystalline material, and run into ethanol (200 c.c.) with efficient stirring and ice-cooling; after being kept overnight, the colourless granular precipitate (25·5 g.) (presumably α-amino*iso*butyramide hydrogen sulphate; cf. Cook and Cox, *loc. cit.*) was collected and washed well with ethanol. This material (23 g.) was suspended in ethanol (100 c.c.) and treated with 2N-methanolic sodium methoxide with vigorous stirring to feeble alkalinity to phenolphthalein. After removal of the sodium sulphate and washing with methanol, evaporation of the filtrate and washings in a vacuum left colourless crystalline α-amino*iso*butyramide (10·5 g., 64% overall). It was purified by sublimation in a high vacuum, leaving only a trace of residue; the m. p. was then 125—126°.

The resublimed amino-amide (1 g.) was dissolved in dried ethyl acetate (10 c.c.) under reflux, carbon disulphide (2 c.c.) added, and heating continued for 2 minutes. On cooling to 0°, the precipitated dithiocarbamate salt solidified, and a further quantity separated during 2 hours at 0°; the colourless salt (1·4 g.), m. p. 110° (indefinite), was collected and washed with ethyl acetate and light petroleum. It was hygroscopic, and decomposed on storage. The salt (1·1 g.) in water (10 c.c.) at 0° was acidified with concentrated hydrochloric acid (2 c.c.), to give crystalline 4 : 4-*dimethyl*-2-*thio*-5-*thiazolidone* (0·45 g., 86% from the dithiocarbamate), m. p. 130—132°; on crystallisation from benzene–light petroleum (1 : 1), the compound formed thick, colourless, rectangular tablets, m. p. 132° (Found: C, 37·5; H, 4·5; N, 8·7. $C_5H_7ONS_2$ requires C, 37·3; H, 4·4; N, 8·7%).

Action of Morpholine on 4 : 4-*Dimethyl*-2-*thio*-5-*thiazolidone*.—The compound (0·2 g.) in warm dry ethyl acetate (10 c.c.) was treated with morpholine (0·5 g.). After reaction had proceeded for 5 minutes at *ca.* 70°, crystallisation was induced by scratching the flask; on slow cooling, a mass of colourless prisms (0·5 g.) was produced. The *morpholinium* 2-*formomorpholidyl*-2-*propyldithiocarbamate* had m. p. 128—129° (foaming) (Found: C, 46·4; H, 7·1; N, 12·4. $C_{12}H_{22}O_3N_3S_2$ requires C, 46·5; H, 7·5; N, 12·5%). On attempted recrystallisation of this salt, decomposition occurred; it was complete after 15 minutes' refluxing in ethyl acetate–methanol (1 : 1), the precipitated crystals, which sublimed at 180°, proving to be morpholinium morpholinedithiocarbamate (Found: C, 42·9; H, 7·4; N, 10·9. Calc. for $C_9H_{14}O_2N_2S_2$: C, 43·2; H, 7·2; N, 11·2%). The original morpholine salt (200 mg.) in water (1 c.c.) was acidified with dilute hydrochloric acid to give the thiothiazolidone (90 mg., 93%), m. p. 129—130° undepressed by admixture with the original sample.

4-n-*Butyl*-2-*thio*-5-*thiazolidone*.—2-Bromo-*n*-hexanoyl bromide (45 g.) (Auwers and Wegener, *J. pr. Chem.*, 1923, **106**, 226) was added cautiously with ice-cooling to saturated methanolic ammonia (500 c.c.). The solution was heated in an autoclave for 24 hours at 70°, and evaporated to dryness in

a vacuum; the residue was suspended in ethanol, and methanolic sodium methoxide was added until the solution was just alkaline to phenolphthalein. The solvents were removed in a vacuum and the dry residue was extracted with hot chloroform (3 × 25 c.c.); on evaporation of the chloroform, the crude amino-amide (20 g.) formed a pasty solid. By sublimation in high vacuum, DL-norleucine amide was obtained as a hard colourless mass (16·1 g., 71%), m. p. 88—90°. It crystallised from *cyclo*hexane in felted, colourless, hygroscopic needles, m. p. 91°, of the ***hemihydrate*** (Found: N, 20·7. $C_6H_{14}ON_2,\frac{1}{2}H_2O$ requires N, 20·1%). The compound was soluble in water, ethanol, acetone, and chloroform, sparingly soluble in ethyl acetate and benzene, and almost insoluble in ether and light petroleum. It was characterised as the ***hydrochloride***, which crystallised in colourless, felted needles, m. p. 228—229°, from methanol–acetone (Found: N, 16·5. $C_6H_{13}ON_2Cl$ requires N, 16·8%).

The amino-amide (12 g.) in dried ethyl acetate (200 c.c.) was treated with carbon disulphide (8 g.); after 1 hour at 0°, the precipitated dithiocarbamate salt (14 g., 90%) was collected, dried in a vacuum, and dissolved in water (300 c.c.) at 0°. Acidification with concentrated hydrochloric acid (25 c.c.) gave 4-n-*butyl*-2-*thio*-5-*thiazolidone* (6·8 g., 86%), m. p. 65°; on sublimation in high vacuum a trace of colouring matter was removed, and subsequent crystallisation from *cyclo*hexane gave colourless hexagonal prisms, m. p. 73—74° (Found: N, 7·4. $C_7H_{11}ONS_2$ requires N, 7·4%).

4-*Carbamyl*-2-*thio*-5-*thiazolidone*.—Aminomalonic ester was prepared from ethyl malonate according to Bentley (Ph.D. Thesis, London, 1945; cf. Cerchez, *Bull. Soc. chim.*, 1930, **47**, 1287) and converted by treatment with aqueous ammonia into the diamide (Piloty and Neresheimer, *Ber.*, 1906, **39**, 514). For conversion into the thiothiazolidone two methods were used. (*a*) Aminomalonamide (5·9 g.), 2N-sodium carbonate (25 c.c., 1 equiv.), and carbon disulphide (5 c.c.) were shaken together at room temperature until all the amide was dissolved (3 days). On acidification with hydrochloric acid, hydrogen sulphide was evolved, and 4-*carbamyl*-2-*thio*-5-*thiazolidone* (1·5 g., 17%) was precipitated as a pale yellow, microcrystalline powder, which gave colourless needles, m. p. 190° (decomp.), after several recrystallisations from acetic acid, although prolonged boiling caused decomposition (Found: C, 27·7; H, 2·55; N, 15·8. $C_4H_4O_2N_2S_2$ requires C, 27·3; H, 2·3; N, 15·9%). The thiothiazolidone was sparingly soluble in the cold in methanol, ethanol, and acetic acid, and insoluble in other common organic solvents and in water. With ferric chloride in aqueous ethanol it gave an intense indigo-blue colour, becoming purple on dilution. When dissolved in hot water, it lost carbon disulphide and carbon dioxide with effervescence, leaving a solution of glycine amide (the presence also of glycine was shown by paper chromatography). (*b*) Aminomalonamide (5 g.) was powdered finely and suspended in pyridine under reflux. Carbon disulphide (10 c.c.) was added cautiously, and refluxing was continued for 5 minutes (longer heating caused loss of hydrogen sulphide, with formation of a water-insoluble, sulphur-containing product, not further examined). On cooling, the colourless crystalline dithiocarbamate salt (3·6 g.) separated, and a further quantity remained on evaporation of the pyridine mother-liquor in a vacuum. The combined crops were dissolved in water (25 c.c.) at 0°, and acidified with ice-cold concentrated hydrochloric acid (10 c.c.) to yield the colourless crystalline thiothiazolidone (3·4 g.; 90% from aminomalonamide), m. p. 189° (decomp.).

Action of Bases on 4-*Carbamyl*-2-*thio*-5-*thiazolidone*.—Compounds with bases were readily produced by addition of the base (1—2 mols.) to the thiothiazolidone (saturated solution) in methanol, followed by dilution with ether. They formed colourless prisms, m. p. (aniline) 128—129° (decomp.), (benzylamine) 138—139° (decomp.), (morpholine) 181° (decomp.). The last *compound* crystallised from methanol in colourless rectangular tablets, m. p. 181° (Found: C, 37·0; H, 4·7; N, 15·9. $C_4H_4O_2N_2S_2,C_4H_9ON$ requires C, 36·5; H, 5·0; N, 15·95%). All the compounds were readily soluble in water, acidification yielding a crystalline precipitate, m. p. 190° (decomp.) undepressed on admixture with the thiothiazolidone. They also gave the same intense blue colour with ferric chloride as the parent compound.

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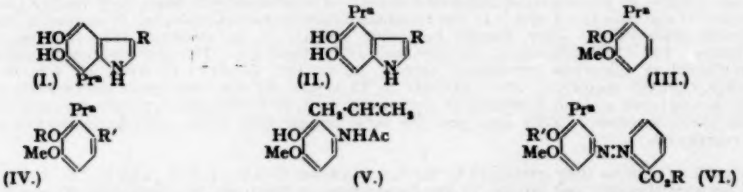
536. *The Chemistry of the Melanins. Part IV.* Some Dihydroxyindoles substituted in the Benzene Nucleus.*

By R. J. S. Beer, J. P. Brown, and Alexander Robertson.

5 : 6-Dihydroxy-7-*n*-propyl- and 5 : 6-dihydroxy-3-methyl-7-*n*-propyl-indole, together with compounds regarded as 5 : 6-dihydroxy-4-*n*-propyl- and 5 : 6-dihydroxy-3-methyl-4-*n*-propyl-indole, have been synthesised in continuation of studies on melanin formation. In their behaviour on autoxidation in faintly alkaline solution, these indoles resemble 5 : 6-dihydroxy-3-methylindole rather than 5 : 6-dihydroxyindole.

In a recent paper (*J.*, 1951, 703) Bu'Lock and Harley-Mason have elaborated a theory of melanin-formation from 5 : 6-dihydroxyindole by way of the corresponding *o*-quinone which is considered to undergo a self-condensation involving the 3- and the 7-positions. A test of the validity of this theory, it is stated, " would be provided by the investigation of the oxidation of a 5 : 6-dihydroxyindole substituted in the 7-position, which should not give a melanin." Two indoles of this type, *viz.*, 5 : 6-dihydroxy-7-*n*-propyl- (I; R = H) and 5 : 6-dihydroxy-3-methyl-7-*n*-propylindole (I; R = Me), and also two compounds believed to be 5 : 6-dihydroxy-4-*n*-propylindole (II; R = H) and its 3-methyl derivative (II; R = Me) were prepared in these laboratories in 1949.† The present communication deals with the synthesis and properties of these compounds.

The chief problem in the synthesis of dihydroxyindoles containing alkyl substituents in the benzene residue is the accessibility of suitable starting materials. Our primary objective was the preparation of 3-alkyl-4-amino- and 3-alkyl-5-amino-veratroles which were to be converted into 5 : 6-dihydroxyindoles by the method employed for the synthesis of 5 : 6-dihydroxy-3-methylindole (Part II, *J.*, 1949, 2061). *o*-Vanillin appeared to be a suitable starting material but, as this compound was not readily available in quantity, we decided to employ 6-*n*-propylguaiacol (III; R = H) which is easily obtained by rearrangement of *O*-allylguaiacol (Claisen and Eisleb, *Annalen*, 1913, **401**, 21) followed by hydrogenation with hydrogen and a palladium–charcoal catalyst (cf. Kurosawa, *Ber.*, 1915, **48**, 1603).



Although 3-methylveratrole on nitration gives 3-methyl-5-nitroveratrole in reasonable yield (Cain and Simonsen, *J.*, 1914, **105**, 156) it was found, in agreement with Kurosawa (*loc. cit.*), that 3-*n*-propylveratrole gave an intractable mixture of nitration products. 6-*n*-Propylguaiacyl acetate (III; R = Ac) also yielded a mixed product, but a pure mononitro-derivative, subsequently shown to be the 5-nitro-derivative (IV; R = Ac, R′ = NO_2), was separated by careful crystallisation. 4-Amino-3-*n*-propylveratrole was then obtained by successive deacetylation, methylation, and catalytic reduction. The base, which was extremely sensitive to aerial oxidation, was isolated as its hydrochloride and characterised by the formation of an acetyl derivative. The same 4-acetamido-3-*n*-propylveratrole was obtained by an independent route which, considered in conjunction with the above, serves to establish its orientation. 5-Nitroguaiacol (Paul, *Ber.*, 1906, **39**, 2773) was converted into 5-acetamidoguaiacol, the allyl ether of which yielded a *C*-allyl derivative (V) on Claisen rearrangement. This compound on methylation and catalytic reduction afforded an acetamido-*n*-propylveratrole identical with that already prepared. Attempts to effect a Claisen rearrangement with the allyl derivative of 5-nitroguaiacol were unsuccessful.

* Part III, *J.*, 1951, 2029.

† The syntheses of these indoles were described in a Thesis presented for the Degree of Doctor of Philosophy of this University by one of us (J. P. B.) in 1949.

4-Amino-3-*n*-propylveratrole was diazotised and condensed with ethyl α-acetylbutyrate, and the resulting oily red hydrazone cyclised to ethyl 5 : 6-dimethoxy-3-methyl-7-*n*-propylindole-2-carboxylate which, on hydrolysis, yielded the corresponding acid. Demethylation was effected with aluminium bromide in boiling toluene and the resulting 5 : 6-dihydroxy-3-methyl-7-*n*-propylindole-2-carboxylic acid, on decarboxylation, gave 5 : 6-dihydroxy-3-methyl-7-*n*-propylindole (I; R = Me). When ethyl α-acetylbutyrate was replaced by ethyl α-acetylpropionate in the initial Japp–Klingemann reaction, a similar sequence of reactions afforded ethyl 5 : 6-dimethoxy-7-*n*-propylindole-2-carboxylate, 5 : 6-dihydroxy-7-*n*-propylindole-2-carboxylic acid, and 5 : 6-dihydroxy-7-*n*-propylindole (I; R = H).

During explorative experiments, 5-*n*-propylvanillin was prepared, in low yield, from 6-*n*-propylguaiacol by application of the Reimer–Tiemann reaction, and also from 5-allylvanillin (Claisen and Eisleb, *loc. cit.*) by catalytic reduction. Methylation of 5-*n*-propylvanillin gave 5-*n*-propylveratraldehyde.

For the preparation of 5-amino-3-*n*-propylveratrole, diazotised methyl anthranilate was condensed with 6-*n*-propylguaiacol, and the resulting azo-compound (VI; R = Me, R′ = H) methylated and then hydrolysed to give the acid (VI; R = H, R′ = Me), a procedure which was more convenient than direct condensation with diazotised anthranilic acid followed by methylation. Hydrogenation of the azo-acid with the aid of a palladium catalyst furnished 5-amino-3-*n*-propylveratrole. This base which, unlike the isomeric 4-amino-3-*n*-propylveratrole, was relatively stable towards atmospheric oxygen, was then converted without difficulty into compounds regarded as 5 : 6-dihydroxy-4-*n*-propylindole (II; R = H) and 5 : 6-dihydroxy-3-methyl-4-*n*-propylindole (II; R = Me). In the synthesis of these compounds there is some ambiguity since the cyclisations may have led to 4 : 5-dihydroxyindole derivatives, but the normal cyclisation leading to 5 : 6-dihydroxyindoles is considered the more probable.

The behaviour of the four dihydroxyindoles on autoxidation in faintly alkaline solution has been studied qualitatively and is described in the Experimental section. All very rapidly form blue or violet precipitates which, in the case of 5 : 6-dihydroxy-7- and 5 : 6-dihydroxy-4-*n*-propylindole, become virtually black in a few hours. The blackening process is apparently very much retarded by the presence of the 3-methyl group in the other indoles.

It has now become clear that the rather crude methods used hitherto for comparison of the autoxidations are inadequate. Superficially, the behaviour of 5 : 6-dihydroxy-3-methylindole (Part II, *loc. cit.*) and of the indoles now described differs from that of 5 : 6-dihydroxyindole itself. Nevertheless, all these indoles give insoluble polymeric products and, although these polymers may well be of different types, in our opinion the differences are not at present sufficiently well-defined to warrant the construction of an elaborate theory of melanin formation. For this reason a more rigorous comparison of the behaviour of the various dihydroxyindoles on autoxidation, and also on enzymic oxidation, is in progress in these laboratories. The results of these studies will be reported later.

Experimental.

6-n-*Propylguaiacol*.—A solution of 6-allylguaiacol (20 g.) in methanol (200 ml.) was agitated with a palladium–charcoal catalyst in an atmosphere of hydrogen (room temperature and pressure) until the theoretical amount of hydrogen was absorbed (*ca.* 10 minutes). Purified by distillation, the product (b. p. 145°/25 mm.) was a colourless oil (19 g.) which, on being cooled, formed colourless needles, m. p. 15° (cf. Kurosawa, *loc. cit.*). **6-n-*Propylguaiacyl* p-*nitrobenzoate*** separated from methanol in colourless prisms, m. p. 96° (Found: C, 64·5; H, 5·5; N, 4·7. $C_{17}H_{17}O_5N$ requires C, 64·8; H, 5·4; N, 4·4%).

O-*Acetyl*-5-*nitro*-6-n-*propylguaiacol*.—Acetylation of 6-*n*-propylguaiacol (20 g.) with acetic anhydride (20 ml.) and concentrated sulphuric acid (2 drops) during 1 hour gave the acetyl derivative (24·1 g.; b. p. 144°/20 mm.) which was purified by distillation. This product (3 g.), dissolved in glacial acetic acid (3 ml.) was treated with a solution of fuming nitric acid (5 ml.; *d* 1·5) in acetic acid (8 ml.) and, when the initial vigorous reaction had subsided, the red solution was poured into ice-water (75 ml.). The sticky solid, obtained by cooling the precipitated oil in a freezing mixture, was collected and crystallised from the minimum quantity of methanol giving the *nitro*-compound (1·2 g.) in very pale yellow prisms, m. p. 92° (Found: C, 56·7; H, 5·7; N, 5·4. $C_{12}H_{15}O_5N$ requires C, 56·9; H, 5·9; N, 5·5%). Nitration of larger batches of *O*-acetyl-6-*n*-propylguaiacol gave less satisfactory results.

5-*Nitro*-6-n-*propylguaiacol*.—*O*-Acetyl-5-nitro-6-*n*-propylguaiacol (12·3 g.) was heated on the steam-bath with a solution of sodium hydroxide (16 g.) in water (120 ml.) for 2 hours. On acidification, the resulting clear red solution deposited 5-*nitro*-6-n-*propylguaiacol* (10·7 g.) forming pale yellow needles, m. p. 77·5°, from 50% aqueous methanol, or colourless needles, m. p. 77·5°, from light petroleum (b. p. 60—80°) (Found: C, 56·8; H, 6·1; N, 6·4. $C_{10}H_{13}O_4N$ requires C, 56·9; H, 6·2; N, 6·6%).

4-*Nitro*-3-n-*propylveratrole*.—When 5-nitro-6-*n*-propylguaiacol (10·2 g.) was added to a solution of sodium hydroxide (4 g.) in water (10 ml.), the bright red sodium phenoxide separated. Methyl sulphate (12 ml.) was then added in portions (1 ml.) with vigorous shaking and, after addition of a further quantity of sodium hydroxide (4 g.) dissolved in water (10 ml.), the mixture was warmed on the

steam-bath for 30 minutes, cooled, and diluted with water (50 ml.). Crystallised from aqueous methanol, the yellow granular product (10·4 g.) gave 4-*nitro*-3-n-*propylveratrole* in long pale yellow needles, m. p. 40° (Found : C, 58·8; H, 6·7; N, 6·4. $C_{11}H_{15}O_4N$ requires C, 58·7; H, 6·7; N, 6·2%).

4-*Amino*-3-n-*propylveratrole*.—A solution of the foregoing nitroveratrole (5 g.) in methanol (100 ml.) was shaken in hydrogen with 20% palladium–charcoal for 2 hours, filtered, and evaporated, giving a purple oil which did not solidify. Since the aminoveratrole was very sensitive to oxidation, it was normally isolated as the hydrochloride (colourless plates, m. p. 195°, with previous darkening). A sample of the amine was converted in good yield into its *acetyl* derivative which formed silky colourless needles, m. p. 140°, from water (Found : C, 65·6; H, 7·9; N, 6·0. $C_{13}H_{19}O_3N$ requires C, 65·8; H, 8·0; N, 5·9%).

5 : 6-*Dimethoxy*-3-*methyl*-7-n-*propylindole*-2-*carboxylic Acid*.—The solution of the diazonium salt prepared from 4-amino-3-*n*-propylveratrole hydrochloride (5 g.), concentrated hydrochloric acid (15 ml.), water (50 ml.), and sodium nitrite (2 g.) was added immediately to a mixture of ethyl α-acetylbutyrate (Michael, *J. pr. Chem.*, 1905, **72**, 553) (4·5 g.) dissolved in 95% ethanol (50 ml.) at 0°, and sodium hydroxide (8 g.) dissolved in ice-water (12 ml.). After having been kept for 1 hour at 0°, the precipitated oily hydrazone was isolated with ether. A rapid stream of dry hydrogen chloride was passed through a solution of the crude hydrazone (7·2 g.) in dry ethanol (70 ml.) for 30 minutes. *Ethyl* 5 : 6-*dimethoxy*-3-*methyl*-7-n-*propylindole*-2-*carboxylate* was then isolated with ether after addition of water (200 ml.). Crystallised from 50% aqueous methanol (charcoal), this ester formed almost colourless needles (1·7 g.), m. p. 106° (Found : C, 66·7; H, 7·4; N, 4·7. $C_{17}H_{23}O_4N$ requires C, 66·9; H, 7·5; N, 4·6%), which gave a green Ehrlich reaction only on warming. The resinous 5 : 6-*dimethoxy*-3-*methyl*-7-n-*propylindole*-2-*carboxylic acid* obtained by hydrolysis of the ester (3 g.) for 1½ hours with boiling 10% alcoholic potassium hydroxide (60 ml.) was triturated with methanol and then crystallised from the same solvent, forming colourless needles (2·3 g.), m. p. 156—157° (Found : C, 64·9; H, 7·1; N, 5·3. $C_{15}H_{19}O_4N$ requires C, 65·0; H, 6·9; N, 5·1%).

5 : 6-*Dihydroxy*-3-*methyl*-7-n-*propylindole*.—A mixture of 5 : 6-dimeth.oxy-3-methyl-7-*n*-propylindole-2-carboxylic acid (0·5 g.), toluene (30 ml.), and aluminium bromide (2·85 g.) was heated under reflux for 25 minutes, cooled to 0°, and agitated with ice-cold dilute hydrochloric acid (50 ml.) and peroxide-free ether. The separated organic layer was combined with further ethereal extracts of the aqueous layer and evaporated, leaving a pearl-grey solid (0·25 g.), m. p. 190—192° (violent decomp.), which gave a deep green Ehrlich reaction at room temperature and a deep blue ferric reaction in water. It dissolved in very dilute aqueous sodium hydrogen carbonate to a blue solution which deposited a blue precipitate after *ca.* 10 minutes.

The crude dihydroxyindole acid (0·2 g.) was decarboxylated by distillation *in vacuo* (1 mm.); the air-bath temperature was raised from 120° to 200° during 10 minutes. The yellow glass which distilled, mainly at *ca.* 160°, partly solidified and after resublimation [120—130° (bath)/0·3 mm.] was crystallised from ligroin, giving 5 : 6-*dihydroxy*-3-*methyl*-7-n-*propylindole* in shining colourless prisms, m. p. 99—100° (Found : C, 70·3; H, 7·4; N, 6·9. $C_{13}H_{15}O_2N$ requires C, 70·2; H, 7·3; N, 6·8%). This indole, which darkens rapidly on exposure to air, gives a purple solution with a strong greenish-blue tinge in the Ehrlich test, and a blue precipitate with aqueous ferric chloride. In dilute aqueous sodium hydrogen carbonate, it forms a blue solution immediately which deposits a blue precipitate within 3 minutes. No further marked change was apparent even after several days. The freshly prepared blue precipitate dissolved momentarily in alcohol to a blue solution which rapidly deposited the pigment again in a highly insoluble form.

5 : 6-*Dihydroxy*-7-n-*propylindole*.—*Ethyl* 5 : 6-*dimethoxy*-7-n-*propylindole*-2-*carboxylate* (1·7 g.) was prepared from 4-amino-3-*n*-propylveratrole hydrochloride (5·1 g.) and ethyl α-acetylpropionate (3·5 g.) (Michael, *Ber.*, 1905, **38**, 2091) by the method described above for the 3-methyl derivative. The ester separated from a little methanol in stout prisms, m. p. 95—96° (Found : C, 66·2; H, 7·3; N, 5·0. $C_{16}H_{21}O_4N$ requires C, 66·0; H, 7·2; N, 4·8%), and gave a permanganate pink colour with hot Ehrlich's reagent. Hydrolysis with boiling 10% alcoholic potassium hydroxide furnished the corresponding *acid* (0·8 g.) which formed colourless prisms, m. p. 164°, from benzene (Found : C, 63·8; H, 6·3; N, 5·2. $C_{14}H_{17}O_4N$ requires C, 63·9; H, 6·5; N, 5·3%). This acid (0·5 g.) was demethylated by the method used for its 3-methyl homologue, yielding 5 : 6-dihydroxy-7-*n*-propylindole-2-carboxylic acid (0·21 g.) as a pale grey amorphous solid, m. p. 180—182° (decomp.), which was decarboxylated directly by being heated *in vacuo* (170°/0·2 mm.) for 30 minutes. Crystallised from ligroin the resulting yellow glass (0·04 g.) gave 5 : 6-*dihydroxy*-7-n-*propylindole* in colourless needles, m. p. 106—107° (Found : C, 69·2; H, 6·8; N, 7·2. $C_{11}H_{13}O_2N$ requires C, 69·1; H, 6·8; N, 7·3%). This indole resembles its homologue in giving a strong purple Ehrlich reaction and a blue-green ferric reaction with subsequent separation of a dark violet precipitate. The violet solution of the indole in dilute aqueous sodium hydrogen carbonate almost immediately deposits a blue-violet precipitate which darkens during a few hours.

5-*Acetamidoguaiacol*.—Reduction of 5-nitroguaiacol (Paul, *Ber.*, 1906, **39**, 2773) (3 g.), dissolved in methanol (50 ml.), with hydrogen (ordinary temperature and pressure) and 20% palladium–charcoal (1 g.) gave 5-aminoguaiacol, which was obtained as pale straw-coloured plates on evaporation of the filtered reaction mixture *in vacuo*. This base darkened rapidly on exposure to air and was, therefore, immediately acetylated with acetic anhydride (10 ml.) and water (30 ml.). Recrystallised from water (charcoal) the resulting 5-acetamidoguaiacol formed shining colourless prisms (2·1 g.), m. p. 171—172° (Jacobs and Heidelberger, *J. Amer. Chem. Soc.*, 1917, **39**, 2195, give m. p. 169—172°) (Found : C, 59·9; H, 6·3; N, 7·9. Calc. for $C_9H_{11}O_3N$: C, 59·7; H, 6·1; N, 7·7%).

5-*Acetamido*-6-*allylguaiacol*.—5-Acetamidoguaiacol (2 g.) in acetone (20 ml.) was heated under reflux for 5 hours with allyl bromide (3·5 ml.) and potassium carbonate (6 g.). The *allyl ether* (2·1 g.)

separated from water in fluffy colourless needles, m. p. 110° (Found: C, 65·0; H, 6·7; N, 6·4. $C_{12}H_{15}O_3N$ requires C, 65·2; H, 6·8; N, 6·3%). Rearrangement to *5-acetamido-6-allylguaiacol* was effected by heating the ether (1·5 g.) initially to 170° and then raising the temperature to 250° during 10 minutes. The solidified melt was crystallised from 50% aqueous methanol and then from benzene, forming large pale pink needles (1·2 g.), m. p. 159° (Found: C, 65·3; H, 6·9; N, 6·1. $C_{12}H_{15}O_3N$ requires C, 65·2; H, 6·8; N, 6·3%).

4-Acetamido-3-n-propylveratrole.—Prepared from the foregoing guaiacol (1 g.) by the methyl iodide–potassium carbonate method, *4-acetamido-3-allylveratrole* (1 g.) crystallised from a small volume of ether in colourless prisms, m. p. 140—141°, or from water in colourless needles, m. p. 141° (Found: C, 66·2; H, 7·4; N, 5·9. $C_{13}H_{17}O_3N$ requires C, 66·4; H, 7·2; N, 6·0%). Hydrogenation of this compound with the aid of a palladium–charcoal catalyst in methanol gave an almost quantitative yield of 4-acetamido-3-*n*-propylveratrole, obtained in colourless silky needles, m. p. 140°, undepressed by admixture with a sample prepared by the alternative route from 6-*n*-propylguaiacol; mixed with 4-acetamido-3-allylveratrole, m. p. 141°, it melted at 137—138°.

5-Amino-3-n-propylveratrole.—Methyl anthranilate (4 g.), dissolved in an ice-cold mixture of concentrated sulphuric acid (4 ml.) and water (20 ml.), was diazotised by addition of sodium nitrite (3 g.) in water (12 ml.). Fifteen minutes later the solution was added in a thin stream to a solution of 6-*n*-propylguaiacol (4 g.) in 10% aqueous sodium hydroxide (80 ml.) at 0°. After having been kept for 10 minutes the clear red solution was acidified and the precipitated azo-dye collected immediately (quantitative yield). Crystallised first from a little methanol and then from ligroin, *methyl 4-hydroxy-3-methoxy-5-n-propylazobenzene-2'-carboxylate* formed small orange prisms, m. p. 150—151° (Found: C, 65·8; H, 6·3; N, 8·9. $C_{18}H_{20}O_4N_2$ requires C, 65·9; H, 6·1; N, 8·5%). Methylation of the crude azo-compound (15 g.) by the methyl iodide–potassium carbonate method in boiling acetone for 12 hours gave the dimethoxy-ester as a red oil which was hydrolysed with boiling 5% methanolic potassium hydroxide for one hour. The resulting 3 : *4-dimethoxy-5-n-propylazobenzene-2'-carboxylic acid* (9·4 g.) was crystallised from a little methanol and then from benzene, forming orange prisms, m. p. 111° (Found: C, 65·8; H, 6·3; N, 8·8. $C_{18}H_{20}O_4N_2$ requires C, 65·9; H, 6·1; N, 8·5%). Reductive fission of this azo-compound (5 g.) in methanol (100 ml.) with a palladium–charcoal catalyst and hydrogen at ordinary temperature and pressure gave *5-amino-3-n-propylveratrole* (2·3 g.) which was precipitated from the concentrated reaction mixture by addition of 2N-sodium hydroxide solution and crystallised from light petroleum, forming clusters of almost colourless needles, m. p. 66·5° (Found: C, 67·4; H, 8·9; N, 7·2. $C_{11}H_{17}O_2N$ requires C, 67·7; H, 8·7; N, 7·2%). The amine was more stable in air than 4-amino-3-*n*-propylveratrole. *5-Acetamido-3-n-propylveratrole* formed colourless needles, m. p. 97°, from hot water (Found: N, 6·1. $C_{13}H_{19}O_3N$ requires N, 5·9%).

5 : *6-Dimethoxy-3-methyl-4-n-propylindole-2-carboxylic Acid.*—Application of the Japp–Klingemann reaction to 5-amino-3-*n*-propylveratrole (4 g.) and ethyl α-acetylbutyrate (3·5 ml.) yielded a pure product (1·8 g.) regarded as *ethyl* 5 : *6-dimethoxy-3-methyl-4-n-propylindole-2-carboxylate* in rosettes of colourless needles, m. p. 134—135° (from methanol) (Found: N, 4·7. $C_{17}H_{23}O_4N$ requires N, 4·6%). Hydrolysis of this ester with hot 10% alcoholic potassium hydroxide gave the corresponding *acid* (1·4 g. from 1·6 g. of ester) which crystallised from benzene in colourless plates, m. p. 189°, and with warm Ehrlich's reagent gave a bright blue colour (Found: C, 64·9; H, 7·0; N, 4·8. $C_{15}H_{19}O_4N$ requires C, 65·0; H, 6·9; N, 5·0%).

5 : *6-Dihydroxy-3-methyl-4-n-propylindole.*—Demethylation of the foregoing acid (0·5 g.) with aluminium bromide (2·85 g.) in warm toluene (30 ml.) afforded 5 : 6-dihydroxy-3-methyl-4-*n*-propylindole-2-carboxylic acid (0·21 g.) as an off-white amorphous solid, m. p. 202° (decomp.). On decarboxylation by the standard procedure, the crude acid (0·2 g.) gave 5 : *6-dihydroxy-3-methyl-4-n-propylindole* (0·15 g.) as a pale yellow oil which soon solidified. Crystallised from ligroin, it formed long colourless needles, m. p. 122° after sintering at 118°, which rapidly deteriorate on exposure to air (Found: C, 70·3; H, 7·4; N, 6·9. $C_{12}H_{15}O_2N$ requires C, 70·2; H, 7·3; N, 6·8%). The blue solution obtained by dissolving this indole in dilute sodium hydrogen carbonate solution deposited within 3 minutes a blue precipitate which slowly darkened in the course of several days. In the Ehrlich test transient violet and green colours were followed by a royal blue coloration.

5 : *6-Dihydroxy-4-n-propylindole.*—Prepared from 5-amino-3-*n*-propylveratrole (3·5 g.) and ethyl α-acetylpropionate (3 ml.), *ethyl* 5 : *6-dimethoxy-4-n-propylindole-2-carboxylate* (1·6 g.) crystallised from light petroleum in colourless needles, m. p. 133° (Found: C, 66·2; H, 7·3; N, 4·9. $C_{16}H_{21}O_4N$ requires C, 66·0; H, 7·2; N, 4·8%). On hydrolysis this ester (0·7 g.) yielded the corresponding *acid* (0·6 g.) which formed colourless triangular prisms, m. p. 162—164° (decomp.), from benzene (Found: C, 64·1; H, 6·7; N, 5·5. $C_{14}H_{17}O_4N$ requires C, 63·9; H, 6·5; N, 5·3%). Demethylation of the acid (0·5 g.) under the usual conditions gave 5 : 6-dihydroxy-4-*n*-propylindole-2-carboxylic acid as a cream-coloured amorphous solid (0·36 g.), m. p. 206° (decomp.). This compound (0·2 g.) was decarboxylated by the standard method, affording 5 : *6-dihydroxy-4-n-propylindole* as a pale yellow gum (0·15 g.) which rapidly solidified. Crystallised from a small volume of benzene, the indole formed short needles, m. p. 93—94° (Found: C, 69·0; H, 6·9; N, 7·0. $C_{11}H_{13}O_2N$ requires C, 69·1; H, 6·8; N, 7·3%), having a purple Ehrlich reaction. The blue solution obtained by dissolving the indole in dilute aqueous sodium hydrogen carbonate almost immediately deposited a violet precipitate which became virtually black in a few hours.

5-n-Propylvanillin.—(*a*) A mixture of 6-*n*-propylguaiacol (3 g.), alcohol (24 ml.), water (12 ml.), chloroform (3·5 ml.), and sodium hydroxide (9 g.) was heated under reflux for 1 hour. After removal of the chloroform and alcohol in a vacuum the aqueous residue was acidified with dilute sulphuric acid, giving *5-n-propylvanillin* (0·6 g.), which was isolated with ether. Crystallised from light petroleum (b. p. 60—80°) this aldehyde formed almost colourless prisms, m. p. 56°, having a blue-green ferric reaction in alcohol (Found: C, 67·8; H, 7·4. $C_{11}H_{14}O_3$ requires C, 68·0; H, 7·2%).

(*b*) 5-Allylvanillin (Claisen and Eisleb, *loc. cit.*) (4·1 g.), dissolved in methanol (100 ml.), was shaken with 20% palladium–charcoal (somewhat deactivated by having been kept in suspension in alcohol for 3 weeks) in hydrogen at ordinary temperature and pressure for 1 hour. Evaporation of the filtered solution and crystallisation of the product from light petroleum gave 5-*n*-propylvanillin as colourless prisms, m. p. and mixed m. p. 56°. Attempts to reproduce the conditions of this experiment, in particular the activity of the catalyst, were not entirely satisfactory and the yields varied widely.

5-n-*Propylveratraldehyde*.—Methylation of 5-*n*-propylvanillin (4 g.) with aqueous sodium hydroxide and methyl sulphate gave 5-*n*-propylveratraldehyde as a colourless oil (4·1 g.), b. p. 190°/50 mm., the *semicarbazone* of which separated from aqueous methanol in rosettes of colourless needles, m. p. 85° (Found : C, 58·7; H, 7·3; N, 15·8. $C_{13}H_{19}O_3N_3$ requires C, 58·9; H, 7·2; N, 15·8%).

O-*Allyl*-5-*nitroguaiacol*.—A mixture of 5-nitroguaiacol (7·5 g.), acetone (30 ml.), allyl bromide (12 ml.), and potassium carbonate (23 g.) was heated under reflux for 7 hours, diluted with water, and extracted with ether. The extracts were washed with alkali, dried, and evaporated, leaving the *allyl ether* which separated from light petroleum (b. p. 60—80°) in pale yellow cubes, m. p. 95° (Found : C, 57·3; H, 5·3; N, 6·3. $C_{10}H_{11}O_4N$ requires C, 57·4; H, 5·3; N, 6·7%).

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537. *Elimination Reactions. Part I. Acid-catalysed Enolisation and Substitution Reactions of Ketones.*

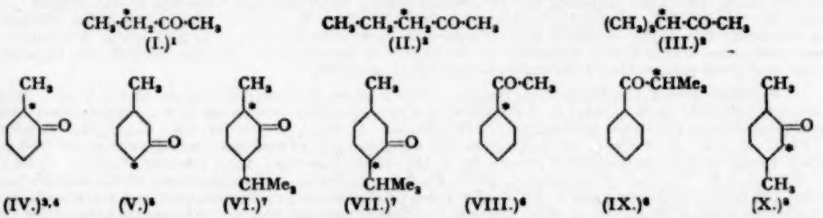
By H. M. E. CARDWELL and A. E. H. KILNER.

Determination of the velocities of production of the isomeric enols from a series of simple alkyl ketones has revealed that orientation of enolisation is controlled by the ability of alkyl groups to be hyperconjugated with the developing double bond in the transition state. The orientation of enolisation largely controls the orientation of substitutions. Rules for the orientation of enolisation are given, and are identical with the modified Saytzeff rule for the dehydrohalogenation of alkyl halides.

There is a pronounced alternation in the hyperconjugative property of *n*-alkyl groups, Me>H; Pr^n>Et; *n*-amyl>Bu^n.

IN the acid-catalysed halogenation of ketones it has been clearly demonstrated (Lapworth, *J.*, 1904, **85**, 30 : Bell, " Acid–Base Catalysis," Oxford Univ. Press, 1941) that the rate-controlling step is the enolisation of the ketone. The orientation of halogenation of unsymmetrical ketones should therefore parallel the orientation of enolisation.

The halogenation of the ketones (I)—(X) is described in the literature; in the majority of cases only the monohalogeno-ketone predominantly formed has been isolated, the asterisks indicating the preferred position of substitution. With the exception of the chlorination of

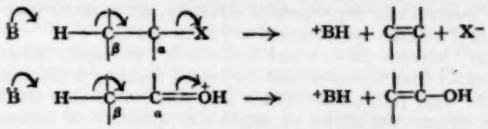


[1] Catch, Elliott, Hey, and Jones, *J.*, 1948, 272. [2] Catch, Hey, Jones, and Wilson, *J.*, 1948, 276. [3] Godchot and Bedos, *Compt. rend.*, 1925, **181**, 919. [4] Borrel and Cornubert, *Bull. Soc. chim.*, 1928, **43**, 804. [5] Godchot and Bedos, *Compt. rend.*, 1924, **178**, 1374. [6] Faworsky, *J. pr. Chem.*, 1913, **88**, 641. [7] Kötz and Steinhorst, *Annalen*, 1911, **379**, 1. [8] Kötz, Blendermann, *et al.*, *Annalen*, 1913, **400**, 47, 55.

2 : 5-dimethyl*cyclo*hexanone (X) all the above halogenations conform to the following rule : " In the acid-catalysed enolisation (halogenation) of an unsymmetrical saturated ketone (acyclic or monocyclic) the proton will be lost most readily from the carbon atom whose adjacent carbon atoms carry the largest number of hydrogen atoms." The rule may be illustrated by 2- and 3-methyl*cyclo*hexanone (IV and V); in (IV) there are five hydrogen atoms on the carbon

atoms adjacent to position 2, and two hydrogen atoms adjacent to position 6; in (V) there are one and two hydrogen atoms respectively adjacent to the corresponding positions: substitution in the 2-position in the former and in the 6-position in the latter is, therefore, expected. In the anomalous case (X) the structure of the product was not conclusively established (see Godchot and Bedos, *loc. cit.*).

The above rule is similar to the extended Saytzeff rule (Dhar, Hughes, Ingold, Mandour, Maw, and Woolf, *J.*, 1948, 2093) for the orientation of dehydrohalogenation of alkyl halides:



The similarity of the two reactions has been noticed by Hughes (*Nature*, 1941, **147**, 813) and by Hauser and Adams (*J. Amer. Chem. Soc.*, 1944, **66**, 345), and the latter have suggested that alkyl substituents must be exerting the same type of influence (Baker–Nathan or hyperconjugative) in both reactions. To test this conclusion, we have determined the proportions of the isomeric monobromo-ketones formed in the acid-catalysed bromination of the following methyl ketones (Pr^i, Bu^n, Bu^i, Bu^s, *n*-amyl, and *n*-hexyl) by the method used by Catch *et al.* (*loc. cit.*) for ethyl methyl and methyl *n*-propyl ketones. Fractional distillation under reduced pressure was used to separate the bromo-ketones, the actual percentages being determined by interpolation on the graph of weight of distillate against boiling point. In all cases the bromomethyl ketones were more lachrymatory and higher-boiling than the bromomethylene ketones. Crystalline derivatives (anilino-compounds from the bromomethyl ketones, and thiazoles from the bromomethylene ketones) were prepared in most cases. Authentic specimens of the bromomethyl ketones (except those from *n*-amyl methyl and *n*-hexyl methyl ketones were prepared (for comparative purposes) by the action of diazomethane followed by hydrogen bromide on the appropriate acid bromides.

The results of these investigations (including the two ketones studied by Catch *et al.*) are given in Table I (cols. ii and iii) together with relative rates of iodination at 25° (col. iv) as determined by Dawson *et al.* (*J.*, 1910, **97**, 2048; *J.*, 1911, **99**, 1740) (the rate figures for *n*-amyl and *sec.*-butyl methyl ketones were kindly provided by Dr. J. Shorter). The itemised rates given in cols. (v)—(viii) are used in the discussion.

It is difficult to estimate the accuracy of these analyses. In all cases, but in particular for *n*-hexyl methyl ketone, the proportion of bromomethyl ketones may have been underestimated. These ketones are sensitive to traces of acids and, as the dibromo-ketones decompose when heated with liberation of hydrogen bromide, it was essential to strip the crude reaction

TABLE I.

		Percentage of:		Rate of iodination ($COMe_2$ = 100).	Rate of formation of enols: >C:C(OH)·CH_3.		>CH·C(OH):CH_2.	
Ser. no.	Ketone. (i.)	>CBr·CO·CH_3. (ii).	>CH·CO·CH_2Br. (iii.)	(iv.)	cols. (ii) × (iv). 100 (v.)	Statistically corrected. (vi.)	cols. (iii) × (iv). 100 (vii.)	Statistically corrected. (viii.)
1	H·CH_2·COMe	50	50	100	50	50	50	50
2	Me·CH_2·COMe	73 *	27	104	76	104	28	28
3	Et·CH_2·COMe	63	37	94	59	89	35	35
4	Pr^n·CH_2·COMe	77	23	110	85	127	25	25
5	Bu^n·CH_2·COMe	60	40	96	58	86	37	37
6	Am^n·CH_2·COMe	80	20	106	85	127	21	21
7	Pr^i·CH_2·COMe	52	48	86	45	67	41	41
8	Me_2CH·COMe	76	24	69	52	156	17	17
9	MeEtCH·COMe	78	22	48·3	38	114	10	10
10	Bu^t·COMe	—	100	46	—	—	46	46
11	Me·CH_2·COEt	50	50	82	41	62	41	62

* Beets (*Rec. Trav. chim.*, 1950, **69**, 307) found 74% of this chloro-ketone in the monochloro-ketones.

mixture from most of the dibromo-ketones before fractionation. In this process significant quantities of the bromomethyl ketones may have been left in the dibromo-ketone fraction. As illustrative of the sensitivity of the bromomethyl ketones we quote three fractionations of

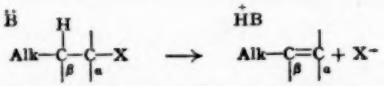
the bromoketones from *n*-amyl methyl ketone. A fractionation at 30 mm. resulted in complete pyrolysis of the bromomethyl fraction, a second fractionation on a new sample at 23 mm. gave 83% of 3-bromoheptan-2-one, but examination of the fraction presumed to be 1-bromoheptan-2-one showed a strong acid reaction, anomalously high refractive index, and abnormally low bromine content. Evidently pronounced decomposition had still occurred. Repetition of the fractionations on a new sample, at 5 mm., gave 60% of the bromomethylene ketone, and both bromo-ketones gave a neutral reaction, normal refractive indices, and excellent analyses. Despite this experimental difficulty, we consider that the analyses (except that for *n*-butyl methyl ketone) are accurate to within $\pm 3\%$.

With the secondary ketones (Nos. 8 and 9, Table I) our results differed so markedly from those of previous workers that we tested and confirmed them by a chemical method of analysis (see p. 2440). Catch, Hey, Jones, and Wilson (*loc. cit.*) stated that the bromination product of methyl *iso*propyl ketone contained no significant quantity of bromomethyl ketone, but examination of their results revealed that they discarded the fraction containing this isomer under the impression that it consisted solely of dibromo-ketones. The discrepancy between our results and those of Bartlett and Stauffer (*J. Amer. Chem. Soc.*, 1935, **57**, 2580) for *sec.*-butyl methyl ketone cannot be resolved so easily. These authors measured the rates of iodination and racemization of the optically active ketone. They concluded that the relative rates of formation of $CMeEt{:}CMe{\cdot}OH$ and $CHMeEt{\cdot}C(OH){:}CH_2$ (overall rate = 100) were 18 and 82, whilst Table I gives 78 and 22, respectively. The two results can only be reconciled if it is assumed that the ketone used by the American authors, $[\alpha]_D^{25} +8{\cdot}15°$, was of very low optical purity.

The results in general are in conformity with our rule. In particular, serial Nos. 2, 3, and 7 illustrate a decrease in percentage of $R{\cdot}CHBr{\cdot}CO{\cdot}CH_3$ as the number of hydrogen atoms on the α-carbon atom in the group R decreases. But it is evident (from serial Nos. 2—6) that other factors are of importance. It is shown below that the full significance of these results only becomes apparent when the percentages are coupled with the rate figures to give the relative rates at which the various enols are formed.

Discussion.

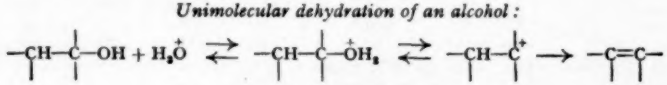
1. *The Mechanistic Basis of Hofmann's and Saytzeff's Rules of Orientation.*—Before analysing the results given in Table I it is necessary to define briefly the factors which determine the type of influence exerted by alkyl substituents in elimination reactions. Hughes and Ingold (*Trans. Faraday Soc.*, 1941, **37**, 657) have concluded that the type of influence exerted by a β-alkyl substituent (see scheme below) depends on whether the β-proton is loosened before, with, or after heterolysis of the C–X bond:



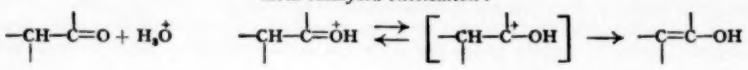
Where X is loosened before the proton, the transition state is highly unsaturated and the alkyl substituent facilitates the elimination reaction by hyperconjugation with the developing double bond; this is the basis of Saytzeff's rule that the most substituted olefin is formed in dehydrohalogenations of alkyl halides. Where, however, the proton is loosened before heterolysis of the C–X bond takes place, the effect of the alkyl substituent is largely inductive or electrostatic, and by increasing the strength of the C–H bond it reduces the rate of elimination; this is the basis of Hofmann's rule that the least substituted olefin is formed in the decomposition of quaternary ammonium hydroxides.

An acid-catalysed enolisation can be written in a manner which is precisely analogous to a unimolecular dehydration of an alcohol:

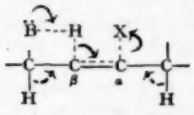
Unimolecular dehydration of an alcohol:



Acid-catalysed enolisation:



In the former reaction (and in the unimolecular elimination reactions of halides and sulphonium salts) the extreme unsaturation of the C^+ sextet results in extreme hyperconjugative control by alkyl substituents. There is, however, an essential difference between the two reactions: in the former, heterolysis of the $C-\overset{+}{O}H_2$ bond is rate controlling whilst, in the latter, the corresponding step is largely imaginary, as $C=\overset{+}{O}H$ and $\overset{+}{C}-OH$ are but two canonical forms of a resonance hybrid to which the former must make much the larger contribution. In acid-catalysed enolisation, therefore, loss of the β-proton and heterolysis of one of the C–O bonds are probably simultaneous (Bell, *op. cit.*). The effect of alkyl substituents on the rate of acid-catalysed enolisation of ketones should therefore be very similar to the effect of such substituents on rates of elimination, where loss of the proton and heterolysis of the C–X bond are known to be simultaneous. The bimolecular dehydrohalogenation of alkyl halides is such a reaction (Hughes and Ingold, *loc. cit.*; Skell and Hauser, *J. Amer. Chem. Soc.*, 1945, **67**, 1661). In this reaction β-alkyl substituents facilitate the elimination by hyperconjugation with the double bond (Dhar, Hughes, Ingold, Mandour, Maw, and Woolf, *J.*, 1948, 2093). In the accompanying diagram (after Dhar *et al.*) the dotted arrows indicate the hyperconjugation or overlap of the C–H bonding orbitals with the developing π-orbital of the double bond. As a C–H orbital overlaps more effectively than a C–C orbital with the developing π-orbital, it is clear that the order of effectiveness of alkyl substituents in facilitating elimination should be Me>Et>Pr^i>Bu^i (3, 2, 1, and 0 C–H bonds, respectively), and that an α-alkyl substituent should be as effective as a β-substituent in facilitating elimination.



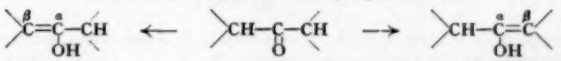
2. *Comparison of Acid-catalysed Enolisation and Bimolecular Dehydrohalogenations.*—A direct comparison of rates of acid-catalysed enolisation and of bimolecular dehydrohalogenations will now be made; for despite the obvious differences between these reactions the effect of alkyl groups on rates of reaction should be very similar in the two cases. The species whose rates of elimination reactions will be compared are $-\overset{|}{C}H-\overset{|}{C}-Br$ and $-\overset{|}{C}H-\overset{|}{C}=\overset{+}{O}H$. The comparison therefore will only be valid if the basicities of the ketones under consideration and hence the concentrations of the oxonium intermediates are approximately the same. The only available evidence (Gordy, *J. Chem. Physics*, 1939, **7**, 93; Gordy and Stanford, *ibid.*, 1940, **8**, 170) suggests that simple alkyl ketones do indeed have approximately the same basicity.

For the purpose of comparison the relative rates at which the enols $CHR{:}CMe{\cdot}OH$ and $CH_2R{\cdot}C(:CH_2){\cdot}OH$ are formed have been calculated from the figures given in Table I in the following manner. Ethyl methyl ketone for instance iodinates at a rate of 104 (acetone = 100); the proportion of $Me{\cdot}CHBr{\cdot}CO{\cdot}CH_3$ in the monobromo-ketone fraction was 73%; the relative rate of formation of $CHMe{:}CMe{\cdot}OH$ is therefore $73 \times 104/100 = 76$, and that of $CH_2Me{\cdot}C(:CH_2){\cdot}OH$ is $27 \times 104/100 = 28$. Relative rates of olefin formation have been calculated in a similar manner from the experimental results of Dhar, Hughes, and Ingold (*J.*, 1948, 2058).

The resulting relative rates are given in Table II (after Dhar *et al.*). In this table the figures on the left are for the rate of production of $\overset{\beta}{\cdot}CH{:}\overset{\alpha}{C}X{\cdot}CH_3$ (X = OH or H) and on the right for $\overset{\alpha}{\cdot}CH_2{\cdot}\overset{\alpha}{C}X{:}\overset{\beta}{C}H_2$; in each case the nature of the alkyl groups on the α- and β-carbon atoms is

TABLE II.

Relative rates of enol and olefin production:



Substituent:						Substituent:		
Serial no.	β.	α.	Rate.	Ketone.	Rate.	α.	β.	Serial no.
1	H	Me	50	Me·COMe	50	Me	H	1
2	Me	Me	(114) 76	Et·COMe	28	Et	H	2
3	Et	Me	(89) 59	Pr·COMe	35	Pr	H	3
4	Me	Et	(62) 41	Et·COEt	41 (62)	Et	Me	4
				Alkyl halide.				
5	H	Me	50	Me·CHBrMe	50	Me	H	5
6	Me	Me	115	Et·CHBrMe	26	Et	H	6
7	Et	Me	80	Pr·CHBrMe	33	Pr	H	7
8	Me	Et	79	Et·CHBrEt	79	Et	Me	8

specified. In addition to the figures for enol-production rate we have given in parentheses figures corrected statistically for the number of hydrogen atoms on the carbon atom from which the proton is lost (*i.e.*, the rate of production of $CH_3{\cdot}CH{:}C(OH){\cdot}CH_3$ is 76. This figure is corrected by multiplying by $\frac{3}{2}$ to allow for the fact that there are three potential protons in the CH_3 group of acetone and only two in the CH_2 group of ethyl methyl ketone. The main justification for this correction (which was first suggested by Dr. Shorter, but see also Bell, Gelles, and Möller, *Proc. Roy. Soc.*, 1949, *A*, **198**, 308) is the extraordinary parallelism that results between the olefin and enol production rates. In effect, we are postulating that where a strong base OH^- is necessary to extract a proton (bimolecular elimination reaction) the probability factor is high, but that where only a weak base (solvent molecule) is necessary (acid-catalysed enolisation) the probability factor is low. [On this reasoning, application of a statistical correction would be appropriate in the unimolecular elimination reaction of alkyl halides, sulphonium salts, etc., where a weak base (solvent molecule) is sufficient to remove the proton.]

The detailed arguments used by Dhar *et al.* for the lower half of the table are repeated below with suitable minor modifications so that they now apply to both halves of the table: "Comparing serial nos. 1, 5 and 2, 6, one sees (left-hand column) that in agreement with theory, the extra β-methyl in 2 and 6 accelerates the formation of $CHMe{:}CXMe$ (X = H or OH) compared with the lower homologue, whereas (right-hand column) the replacement of α-Me by α-Et retards, as it should, the formation of $CEtX{:}CH_2$. Comparing serial nos. 2, 6 and 3, 7, we find (left), again in agreement with theory, that the replacement of a β-Me by β-Et retards the formation of $CHEt{:}CXMe$ compared with $CHMe{:}CXMe$, whereas (right) the replacement of α-Et by α-Pr, which in theory should have little effect, actually slightly accelerates the formation of $CXPr{:}CH_2$ in comparison with $CEtX{:}CH_2$."

Comparing serial nos. 3, 7 and 4, 8 (left) a discrepancy between the two halves of the table is apparent. In the elimination reaction, interchange of Me and Et substituents between the α- and β-positions has no effect on the rate of production of the particular olefins, whereas with the ketones the combination of α-Me–β-Et is more effective than the combination α-Et–β-Me. Such a discrepancy is not unexpected, as the transition state in enol production (where the oxygen atom is still firmly bound to the carbon) must be less symmetrical than the transition state in olefin production (where the bromide ion is only partially bound to carbon). Indeed, serial nos. 3 and 4 still provide convincing evidence that the alkyl groups are acting in a hyperconjugative manner, for if their effect was inductive serial no. 3 would show a lower rate than serial no. 4.

The above analysis establishes the common mechanistic basis of bimolecular dehydrogalogenations and acid-catalysed enolisations, and reveals that our empirical rule of orientation of enolisation reactions is founded on the ability of alkyl groups to facilitate the reaction by hyperconjugation with the developing double bond in the transition state. Orientation according to the Saytzeff rule is more extreme in dehydrohalogenations than in enolisations, for only in the latter is the orientation affected by the number of potential protons available. For instance, although the substituents are acting *quantitatively* in the same manner (see Table II, serial nos. 2 and 6) in the two reactions given below the orientation is more pronounced in the elimination reaction:

$$(73\%)\ CH_3{\cdot}CH{=}\underset{\displaystyle OH}{C}{\cdot}CH_3 \longleftarrow CH_3{\cdot}CH_2{\cdot}\overset{\displaystyle +}{\underset{\displaystyle OH}{C}}{\cdot}CH_3 \longrightarrow CH_3{\cdot}CH_2{\cdot}\underset{\displaystyle OH}{C}{=}CH_2\ (27\%)$$

$$(81\%)\ CH_3{\cdot}CH{=}CH{\cdot}CH_3 \longleftarrow CH_3{\cdot}CH_2{\cdot}\underset{\displaystyle Br}{CH}{\cdot}CH_3 \longrightarrow CH_3{\cdot}CH_2{\cdot}CH{=}CH_2\ (19\%)$$

In the next example (Hughes, Ingold, and Mandour, *J.*, 1948, 2090), we also observe a more pronounced orientation in the elimination reaction:

$$(76\%)\ (CH_3)_2C{=}\underset{\displaystyle OH}{C}{\cdot}CH_3 \longleftarrow (CH_3)_2CH{\cdot}\overset{\displaystyle +}{\underset{\displaystyle OH}{C}}{\cdot}CH_3 \longrightarrow (CH_3)_2CH{\cdot}\underset{\displaystyle OH}{C}{=}CH_2\ (24\%)$$

$$(82\%)\ (CH_3)_2C{=}CH{\cdot}CH_3 \longleftarrow (CH_3)_2CH{\cdot}\underset{\displaystyle I}{CH}{\cdot}CH_3 \longrightarrow (CH_3)_2{\cdot}CH{\cdot}CH{=}CH_2\ (18\%)$$

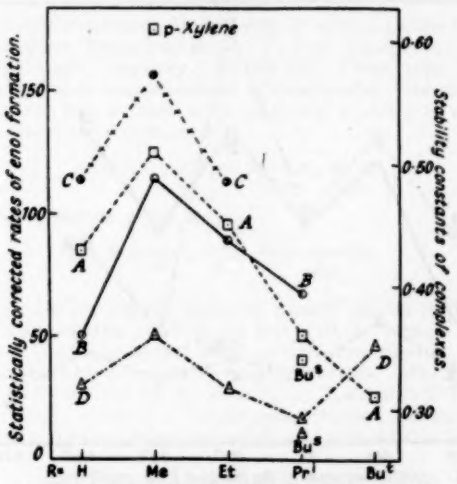
When the necessary statistical correction is applied to the methyl *iso*propyl ketone figures, the expected proportion of $(CH_3)_2C{:}CH{\cdot}CH_3$ in the olefin mixture becomes $\frac{76 \times 3}{76 \times 3 + 24} \times 100 = 90\%$—significantly higher than that observed. Hence, paradoxically, although the Saytzeff

orientation is more extreme in the elimination reaction, the substituents are acting in a more extreme hyperconjugative manner in the enolisation.

3. *Comparison of the Effect of Alkyl Groups on Acid-catalysed Enolisation, and on Degree of Complex Formation between Alkylbenzenes and Picric Acid.*—(a) *Introduction.* A detailed comparison between elimination reactions of alkyl bromides and enolisation reactions of ketones cannot be extended further as no data are available for alkyl bromides corresponding to the other ketones which have been investigated. The comparison with dehydrohalogenations having established that alkyl groups are acting in a hyperconjugative manner in enolisation, there is no longer any need to restrict the comparison to reactions of the same mechanistic type. Instead, any reaction may be selected in which the groups with which we are concerned (Me to n-C_6H_{13}; Pr^i and Bu^t) have been shown to act in a hyperconjugative manner. The only reaction which fulfils these requirements is that between picric acid and alkylbenzenes, which has been studied by Anderson and Hammick (*J.*, 1950, 1089).

The formation of picric acid complexes is dependent on the electron density in the nucleus of the alkylbenzenes. This electron density is increased by (*a*) the inductive electron-release

FIG. 1.



A. Stability constant of the complex PhR–*Picric acid.*
B. Rate of formation of R·CH:C(OH)·CH_3.
C. ,, ,, R·CMe:C(OH)·CH_3.
D. ,, ,, R·C(OH):CH_2.

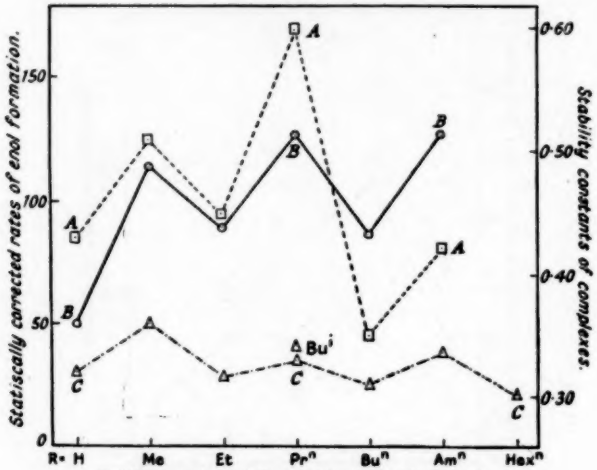
of alkyl groups where $Bu^t > Pr^i > Et > Me > H$, and may be increased (*i.e.*, by polarisation demand of a reagent) by (*b*) the hyperconjugative release of electrons where $Me > Et > Pr^i > Bu^t > H$. The inductive effect should be very little affected by the electron-accepting power of the acid component of the complex. The hyperconjugative effect, however, is a polarisability effect and should vary with the cationoid nature of the acid component. Davies and Hammick (*J.*, 1938, 763) observed that the degree of complex formation between tetranitromethane and PhR varied with R as follows: $R = Bu^t > Et > Me > Pr^i > H$. This order was interpreted as indicative of an inductive effect coupled with a hyperconjugative effect. With the more powerfully cationoid reagent (picric acid) (Anderson and Hammick, *loc. cit.*) the order was $R = Me > Et > H > Pr^i > Bu^t$; with the exception of the position of hydrogen in the series this is the normal hyperconjugative order for the ability of alkyl groups to donate electrons to an unsaturated system. This therefore is clearly a polarisability or hyperconjugative effect.

(b) *Branched alkyl groups.* In Fig. 1 are plotted the statistically corrected rates for the production of CHR:C(OH)·CH_3 (R = H to Pr^i) and for R·C(OH):CH_2 (R = H to Bu^t) (the value for acetaldehyde, 30·4, is a revised figure provided by Dr. J. Shorter, who has asked us to correct that, 38·8, given in error in *J.*, 1950, 3425). In addition, stability constants are

plotted for the complexes PhR–picric acid (R = H to Bu^t). The enolisation rates for variations in the β-group closely parallel the stability constants, and we have no doubt that CHBu^t:C(OH)·CH$_3$ will be formed at a low rate from *tert.*-butylacetone (this will be investigated). With α-groups, however, we note that Bu^t·C(OH):CH$_2$ is formed at an anomalously high rate from pinacone. It is unlikely that this anomaly is due to a high basicity in pinacone.* The anomaly, however, is not peculiar to pinacone, for *tert.*-butyldimethylcarbinyl halides (Bu^t·CMe$_2$·Hal) show an abnormally high rate of unimolecular solvolysis and elimination, the product being largely olefin (Shorter and Hinshelwood, *J.*, **1949**, 2412; Brown and Fletcher, *J. Amer. Chem. Soc.*, **1949**, **71**, 1845; **1950**, **72**, 1223; Hughes, Ingold, Martin, and Meigh, *Nature*, **1950**, **166**, 679). The structural similarity between these halides and pinacone suggests that the same factors, at present unknown, are operative in both cases.

It was noted earlier that methyl *iso*propyl ketone (Me + Me hyperconjugation) gave less bromopropyl ketone (RR'CBr·COMe) than did *sec.*-butyl methyl ketone (Me + Et hyperconjugation). Itemised rate figures resolve this anomaly, for they show that the bromoketone (R = R' = Me), rate 52, is formed considerably faster than the ketone (R = Me, R' = Et), rate 38. The orientation anomaly is therefore due to the high rate of formation of Pr^i·C(OH):CH$_2$, and not, as might be inferred in the absence of rate figures, to a high rate of formation of CMeEt:CMe·OH. The rate of formation of this enol is indeed a little lower than expected, if it is assumed that the introduction of a β-methyl group produces the same effect on passing from CH$_2$Et·COMe (59) to CHEtMe·COMe as it does on passing from CH$_2$Me·COMe (76) to CHMe$_2$·COMe (52). On this basis the calculated rate of formation of CMeEt:CMe·OH is 52 × 59/76 = 40, a little higher than the observed value of 38.

FIG. 2.



A. Stability constant of the complex PhR–*picric acid.*
B. Rate of formation of R·CH:C(OH)·CH$_3$.
C. ,, ,, R·C(OH):CH$_2$.

The above figures are for relative rates; the resulting statistically corrected rates of formation of CRMe:CMe·OH (R = H, Me, Et) are plotted in Fig. 1. The fact that this graph roughly parallels the equivalent graph for CHR:CMe·OH, which it certainly would not in the absence of the statistical correction, supports the earlier suggestion that the electronic effects of β-substituents cannot be rationalized until this correction is applied.

* If pinacone had a higher basicity by virtue of the strong inductive effect of the *tert.*-butyl group than the other ketones investigated in this paper, then COPr$^i_2$ with its two Pr^i groups might also be expected to enolise at a faster rate than might be deduced from the partial rates for acetone and COMePr^i. These partial rates (Table I, Serial Nos. 1 and 8, cols. v and vii) suggest that the rate for COPr$^i_2$ should be 2(52 × 17)/50 = 35. Dr. J. Shorter, however, finds that the rate is 12·6. This suggests either that COPr$^i_2$ has a *weaker* basicity than the other ketones or, far more likely, that the rate of formation of Pr^i·C(OH):CH$_2$ is abnormally large.

(c) n-*Alkyl groups. The Alternation effect.* In Fig. 2, graphs (similar to those in Fig. 1) are given for the effect of *n*-alkyl groups. The similarity between the variations in rates of enolisation and in stability constants of picric acid complexes is remarkable. In both cases a striking alternation in the effect of *n*-alkyl groups, Me>H; Pr^n>Et and Bu^n; Am^n>Bu^n and *n*-C_6H_{13}, is observed. This alternation was not expected at the start of this investigation, but in retrospect we may recall that Dhar *et al.* noted that $CHPr^n{:}CH_2$ was formed more rapidly than $Et{\cdot}CH{:}CH_2$ from the corresponding secondary bromides (Table II, serial nos. 6 and 7). In addition, the very slight alternation in overall rates of iodination of the *n*-alkyl methyl ketones (Table I, col. iv, serial nos. 1—6) might have been noted. The alternation in overall rates is slight because each increase in rate of production of $R{\cdot}\overset{\beta}{C}H{:}\overset{\alpha}{C}Me{\cdot}OH$ (R = Me, Pr^n, *n*-amyl) over the next lower homologue (R = H, Et, Bu^n) is compensated by a decrease in rate of production of $H{\cdot}\overset{\beta}{C}H{:}\overset{\alpha}{C}R{\cdot}OH$ (R = Et, Bu^n, *n*-C_6H_{13}) over the next lower homologue (R = Me, Pr^n, *n*-amyl). That an alternation in overall rate is still observable, despite these compensating alternations in partial rates, is due to the fact that, α- and β-groups being largely equivalent, the combination α-Me–β-R always shows a larger rate than the combination α-R–β-H.

An alternation in the hyperconjugative property of *n*-alkyl groups has been predicted on theoretical grounds (Mulliken, Rieke, and Brown, *J. Amer. Chem. Soc.*, 1941, **63**, 41; Dewar, "Electronic Theory of Organic Chemistry," Oxford Univ. Press, 1949, p. 157). Crudely, we can picture every C–C bond as having an element of unsaturation; the strong hyperconjugative property of a methyl group with its three C–H bonds will therefore be more effectively transmitted by an even number of C–C bonds, as in

$$H_3C\underset{\delta}{-}CH_2\underset{\gamma}{\cdots}CH_2\underset{\beta}{-}CH_2\underset{\alpha}{\cdots}CH_2-\underset{OH}{C}=CH_2$$

than by an odd number of carbon atoms, as in

$$H_3C\underset{\gamma}{-}CH_2\underset{\beta}{-}CH_2\underset{\alpha}{-}CH_2-\underset{OH}{C}=CH_2$$

Such an effect should result in a slightly increased electron density in the α–β and γ–δ bonds, with consequent shortening of these bonds in the first example, whereas in the second example the electron density and bond lengths (α–β and β–γ) should be the same. The effect, however, would probably be too small for detection by existing methods. Where, however, the weakly conjugative H_3C- group is replaced by the strongly conjugative $O{=}C(OH){\cdot}$ group the effect becomes large enough for its detection. For instance, Morrison and Robertson (*J.*, 1949, 980, 987, 993, 1001) found that the bond lengths in adipic acid alternated in length as shown below:

$$O=\underset{OH}{\overset{1\cdot52}{C}}-\underset{\delta}{CH_2}\overset{1\cdot49}{\cdots}\underset{\gamma}{CH_2}-\overset{1\cdot54}{CH_2}-\underset{\beta}{CH_2}\overset{1\cdot49}{\cdots}\underset{\alpha}{CH_2}-\overset{1\cdot52}{\underset{OH}{C}}=O$$

A similar alternation in bond lengths was observed in two other even-numbered dicarboxylic acids, succinic and sebacic. In glutaric acid, however, all the C–C bonds were of the same length as shown below:

$$O=\underset{OH}{\overset{1\cdot53}{C}}-\underset{\gamma}{CH_2}-\overset{1\cdot53}{CH_2}-\underset{\beta}{CH_2}-\overset{1\cdot53}{CH_2}-\underset{\alpha}{CH_2}-\overset{1\cdot53}{\underset{OH}{C}}=O$$

The variations in electron density associated with these variations in bonds length are clearly displayed in the electron contour maps given by Morrison and Robertson.

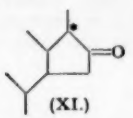
This pronounced difference between the odd- and even-numbered carboxylic acids is probably responsible for the well-known alternation in melting points, the more conjugated acids (even series) having the higher melting points. In conformity with these ideas, the alternation in melting points is much smaller but still observable in the monocarboxylic acids. The analogy which Anderson and Hammick drew between these melting points and the stability constants of picric acid complexes is therefore valid, and these very varied phenomena are all reflections of the fundamental alternation in hyperconjugative properties of *n*-alkyl groups.

This theory of alternation also explains the high rate of formation of $(CH_3)_2CH\cdots CH_2{\cdot}C(OH){:}CH_2$ (Fig. 2), where the effect of both methyl groups is transmitted through the C–C bond.

4. *Other Acid-catalysed Substitution Reactions.*—The general rules which have been developed in this paper can be applied directly to any substitution reaction where the reagent reacts with the enol as soon as it is formed. In reactions where enolisation is not rate-controlling, orientation of substitution will be influenced by the relative concentrations of the isomeric enols at equilibrium. As an alkyl substituent will stabilise the enol in a hyperconjugative manner in the same way as it lowers the energy of the transition state leading to the enol, the concentrations of the enols should be proportional to their rates of formation. (In this respect acid-catalysed substituent reactions are quite different from base-catalysed reactions; see following paper.)

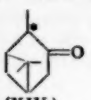
For instance, Shorter and Hinshelwood (*J.*, 1950, 3276) have studied the oxidation of acetone by ceric acid, and Shorter (*J.*, 1950, 3425) has studied this reaction for other aliphatic ketones. Enolisation is a prior step to oxidation, but the enols are not oxidised as soon as they are formed. With higher alkyl ketones oxidation appears to take place almost exclusively on the enol R·C(:)CMe·OH, and the rates of oxidation are proportional to the rates of production of these enols as determined in this paper. This can only mean that the equilibrium concentrations of these enols are proportional to their rates of production, as we have already suggested.

Numerous acid-catalysed oxidations of ketones are recorded by Simonsen and Owen ("The Terpenes," Vols. I and II, Cambridge, 1947 and 1949). The compounds VI, VII, and (XI)—(XIV) undergo oxidation fission between the carbon atom marked * and the carbonyl group.

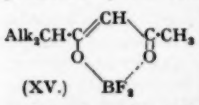

(XI.)

(XII.)

(XIII.)


(XIV.)

In addition, Fileti and Ponzio (*J. pr. Chem.*, 1897, **55**, 186) found that $CH_3Me{\cdot}CO{\cdot}CH_2Pr^i$ on oxidation by nitric acid gave $Me{\cdot}CO{\cdot}CO{\cdot}CH_2Pr^i$, whilst $CH_3Me{\cdot}CO{\cdot}CH_2Et$ gave a mixture of $Me{\cdot}CO{\cdot}CO{\cdot}CH_2Et$ and $CH_3Me{\cdot}CO{\cdot}COEt$. All these results suggest that enolisation, as predicted by our rule, is a prior and orientating step in the oxidation. The orientation of acid-catalysed aldol condensations may also be predicted successfully from our rules. The orientation of acylation of ketones with acetyl chloride or acetic anhydride (catalysed by boron trifluoride) (Hauser and Adams, *loc. cit.*) also follows the Saytzeff-type rule, but with secondary ketones $CHAlk_2{\cdot}CO{\cdot}CH_3$ the reversibility of the reaction and the fact that $CHAlk_2{\cdot}CO{\cdot}CH_2{\cdot}CO{\cdot}CH_3$ can form a complex of the type (XV) (Morgan and Tunstall, *J.*, 1924, **125**, 1963) whilst the isomer $CH_3{\cdot}CO{\cdot}CAlk_2{\cdot}CO{\cdot}CH_3$ cannot, results in more acylation on the CH_3 group than would be expected.


(XV.)

5. *Effect of Other Groups.*—The above arguments * could naturally be extended to ketones containing more polar and unsaturated groups in the same way that Dhar *et al.* have extended their arguments on elimination reactions. Such an extension, however, is complicated by the fact that such groups have marked effects on the basicity of the ketones and in some cases make the halogenation of the enol reversible. A discussion of these topics will therefore be reserved for a later occasion, as will the effect of acid catalysts other than the hydroxonium ion on the orientation of enolisation.

Conclusions.

1. In saturated aliphatic and monocyclic ketones the orientation of acid-catalysed enolisation is controlled by the ability of α- and β-alkyl substituents to stabilise the transition state in a hyperconjugative manner.

2. The orientation of enolisation owing to the operation of the above factor, is slightly obscured by the fact that the rate of enolisation is proportional to the number of potential protons available.

3. The most stable enol is produced most rapidly; hence the orientation of substitution is very largely controlled by the orientation of enolisation, and is almost independent of the substituting reagent, provided that subsequent reactions (*e.g.*, dehydration in the aldol condensation) are equally ready for the two possible primary products.

* All previous arguments applied only to saturated acyclic or monocyclic ketones containing no hetero-atom other than the single carbonyl oxygen atom.

4. As α- and β-substituents exert approximately equal effects, the modified Saytzeff rule given earlier can be further refined and expressed as follows: In the dehydrohalogenation of saturated aliphatic halides and in the acid-catalysed enolisation of saturated aliphatic ketones, the double bond (in olefin or enol) will preferentially be established between those carbon atoms whose adjacent carbon atoms carry the largest number of hydrogen atoms.

5. There is a well-marked alternation in the hyperconjugative ability of *n*-alkyl groups to release electrons: $Pr^n > Et$ and Bu^n, $n\text{-}C_5H_{11} > Bu^n$ and $n\text{-}C_6H_{13}$.

Experimental.

(M.p.s are uncorrected. Analyses are by Drs. Weiler and Strauss and Mr. F. C. Hall.)

Preparation of Ketones.—*n*-Butyl and *sec.*-butyl methyl ketones were prepared by the action of *n*-butyl- and *sec.*-butyl-magnesium bromides on acetic anhydride (Newman and Smith, *J. Org. Chem.*, 1948, **13**, 592). *iso*Butyl methyl ketone was prepared by oxidation of *sec.*-hexyl alcohol. Methyl *iso*propyl ketone was prepared by the method of Whitmore, Evers, and Rothrock (*Org. Synth.*, Coll. Vol. II, p. 408). *n*-Amyl and *n*-hexyl methyl ketones were commercial samples.

The ketones were fractionated through a 60-cm. column (packed with Fenske helices) and had the following physical constants: *n*-Butyl methyl ketone, b. p. 125—126°/760 mm.; *iso*butyl methyl ketone, b. p. 114·5°/744 mm.; *sec.*-butyl methyl ketone, b. p. 115—117°/760 mm.; methyl *iso*propyl ketone, b. p. 93—94°/760 mm., *n*-amyl methyl ketone, b. p. 149·5°/750 mm.; *n*-hexyl methyl ketone, b. p. 169—170°/750 mm.

Bromomethyl ketones. Authentic specimens of some of these ketones were prepared by the action of hydrogen bromide on the diazo-ketones, which were prepared from *n*-valeroyl, *iso*valeroyl, and α-methylbutyryl bromides and diazomethane (Catch, Elliott, Hey, and Jones, *J.*, 1948, 278).

Bromination.—The ketones were brominated at 40—50° with bromine in the presence of potassium chlorate (*idem, ibid.*, p. 272). In each case, the monobromo-ketones were roughly stripped of unchanged ketone and dibromo-ketones by distillation under reduced pressure.

Fractionation of the Bromo-ketones.—The fractionating assembly was a copy of that used by Catch *et al.* (*loc. cit.*), except that the packed section of the column was 60 cm. long. (Drawings of the assembly were kindly provided by Dr. Catch.)

(a) *Monobromo-ketones from* n-*Butyl Methyl Ketone.*—The monobromo-ketones (b. p. 65—125°/45 mm.; 147 g.) on fractionation at 40 mm. (reflux ratio 20 : 1) gave the following fractions (total 112 g.): (i) b. p. to 82·5° (4·8 g.); (ii) b. p. 82·5—85·5° (2·2 g.); (iii) b. p. 85·5—87·5° (58·3 g.); (iv) b. p. 87·5—88·5° (3·1 g.); (v) b. p. 88·5—91·0° (4·8 g.); (vi) b. p. 91—92° (4·2 g.); (vii) b. p. 92·0—93·0° (1·7 g.); (viii) b. p. 93·0—95·0° (2·4 g.); (ix) b. p. 95·0—98·0° (7·4 g.); (x) b. p. 98·0—100·0° (3·8 g.); (xi) b. p 100·0—103·0° (5·8 g.); (xii) b. p. 103·0—106·0 (3·2 g.); (xiii) b. p. 106·0—110·0° (2·2 g.); (xiv) b. p 110·0°/25 mm. (8·5 g.).

Fractions (iii) and (iv) on redistillation gave *3-bromohexan-2-one* (36·8 g.), b. p. 82°/30 mm., n_D^{20} 1·4591 (Found: Br, 44·4. $C_6H_{11}OBr$ requires Br, 44·6%). *2-Amino-4-methyl-5-n-propylthiazole* (prepared from the bromo-ketone and thiourea in 97% yield) crystallised from light petroleum (b. p. 40—60°) in colourless needles, m. p. 45—45·5° (Found: C, 54·1; H, 7·6. $C_7H_{12}N_2S$ requires C, 53·8; H, 7·7%). The *picrate* of the thiazole crystallised from acetone in yellow needles, m. p. 218—220° (decomp.) (Found: C, 40·8; H, 4·0. $C_7H_{12}N_2S,C_6H_3O_7N_3$ requires C, 40·5; H, 3·9%). *3-Anilinohexan-2-one* (prepared from the above bromo-ketone and aniline) was a yellow oil, b. p. 106°/1·0 mm., n_D^{20} 1·5399 (Found: C, 74·6; H, 9·4. $C_{12}H_{17}ON$ requires C, 75·5; H, 9·0%).

Fractions (vi)—(xi) on redistillation gave 1-bromohexan-2-one (13·2 g.), b. p. 96°/30 mm., n_D^{20} 1·4649, analysis (Found: Br, 43·4. Calc. for $C_6H_{11}OBr$: Br, 44·6%) indicating that some decomposition had occurred: our specimen from *n*-valeroyl bromide had b. p. 98—99°/33 mm., n_D^{20} 1·4612 (Catch *et al.* give b. p. 108°/50 mm., $n_D^{15\cdot5}$ 1·4486). *2-Amino-4-n-butylthiazole* (prepared from the above bromo-ketone and thiourea) was a viscous, pale yellow liquid, b. p. 110—111°/1·0 mm., n_D^{21} 1·5515 (Found: C, 53·4; H, 8·0. $C_7H_{12}N_2S$ requires C, 53·8; H, 7·7%). The *picrate* of the thiazole crystallised from acetone in pale yellow needles, m. p. 214—216° (decomp.) (Found: C, 41·0; H, 4·0. $C_7H_{12}N_2S,C_6H_3O_7N_3$ requires C, 40·5; H, 3·9%). *1-Anilinohexan-2-one* crystallised from acetone in colourless needles, m. p. 74·5—75° (Found: C, 75·1; H, 8·8. $C_{12}H_{17}ON$ requires C, 75·5; H, 9·0%).

The thiazole picrate and anilino-compounds did not depress the m. p.s of these compounds prepared from a sample of 1-bromohexan-2-one prepared by the diazomethane method.

(b) *Monobromo-ketones from* iso*Butyl Methyl Ketone.*—The monobromo-ketones (b. p. 60—113°/45 mm.; 268 g.) on fractionation at 60 mm. (reflux ratio 20 : 1) gave the following fractions (total 245 g.): (i) b. p. to 55·0° (21·9 g.); (ii) b. p. 55·0—83·0° (0·25 g.); (iii) b. p. 83·0—88·5° (0·6 g.); (iv) b. p. 88·5—89·0° (7·15 g.); (v) b. p. 89·0—90·5° (70·7 g.); (vi) b. p. 90·5—93·0° (19·1 g.); (vii) b. p. 93·0—96·0° (4·9 g.); (viii) b. p. 96—98° (7·3 g.); (ix) b. p. 98—102° (59·7 g.); (x) b. p. 102—105° (24·7 g.); (xi) b. p. 105—108° (4·55 g.); (xii) b. p. 108—115° (3·5 g.); (xiii) b. p. 115—125° (4·1 g.); (xiv) column stripped at 30 mm. (16·9 g.).

Fractions (v) and (vi) on redistillation gave *3-bromo-4-methylpentan-2-one* (81·1 g.), b. p. 57—59°/13 mm., n_D^{19} 1·4619. This sample gave the folowing b. p.s: 79°/40 mm.; 88°/60 mm.; 95°/80 mm.; 102°/100 mm. (Found: Br, 44·2. $C_6H_{11}OBr$ requires Br, 44·6%). *2-Amino-4-methyl-5-isopropylthiazole* (prepared from the above bromo-ketone and thiourea) crystallised from light petroleum (b. p. 40—60°) in yellow needles, m. p. 72—73° (Found: C, 53·8; H, 7·5. $C_7H_{12}N_2S$ requires C, 53·8; H, 7·7%). The *picrate* crystallised from acetone in pale yellow needles, m. p. 210° (decomp.) (Found:

C, 40·4; H, 3·9. $C_7H_{12}N_2S,C_8H_8O_7N_2$ requires C, 40·5; H, 3·9%). **3-*Benzoyloxy*-4-*methylpentan*-2-*one*** (prepared from the bromo-ketone and potassium benzoate) crystallised from light petroleum (b. p. 40—60°) in colourless needles, m. p. 35—36° (Found : C, 71·0; H, 7·2. $C_{13}H_{16}O_3$ requires C, 70·9; H, 7·2%). A mixture with the isomeric 1-benzoyloxy-4-methylpentan-2-one (m. p. 34—35°, see below) melted at 30°.

Fractions (vii)—(x) on redistillation gave 1-bromo-4-methylpentan-2-one (76·1 g.), b. p. 69—71°/15 mm., n_D^{19} 1·4638. This sample gave the following b. p.s : 94°/40 mm.; 105°/60 mm.; 113·5°/80 mm.; 122°/100 mm. Analysis (Found : Br, 46·0. Calc. for $C_6H_{11}OBr$: Br, 44·6%) indicated that it was contaminated with some dibromo-ketone. A specimen prepared from *iso*valeroyl bromide had b. p. 76—78°/19 mm., n_D^{20} 1·4620. Catch *et al.* (*loc. cit.*) give b. p. 101—102°/50 mm., n_D^{17} 1·4595. A sample of this ketone on treatment with thiourea gave a 97% yield of 2-*amino*-4-iso*butylthiazole*, b. p. 111—112°/1·5 mm., n_D^{20} 1·5441 (Found : C, 54·1; H, 7·6%). The *picrate*, m. p. 201—202° (decomp.) (Found : C, 40·9; H, 4·2. $C_7H_{12}N_2S,C_6H_3O_7N_3$ requires C, 40·5; H, 3·9%), did not depress the m. p. of a sample of the picrate prepared from the thiazole (b. p. 111—112°/1·5 mm., n_D^{20} 1·5438) which in turn had been prepared from authentic 1-bromo-4-methylpentan-2-one. **1-*Anilino*-4-*methylpentan*-2-*one*** (prepared from the bromo-ketone and aniline) crystallised from ether in colourless plates, m. p. 54·5—55° alone or mixed with an authentic specimen prepared *via* the diazo-ketone (Found : C, 75·7; H, 8·9. $C_{12}H_{17}ON$ requires C, 75·5; H, 8·9%). **1-*Benzoyloxy*-4-*methylpentan*-2-*one*** (from the bromo-ketone and potassium benzoate) solidified after distillation, b. p. 154°/5 mm., and then crystallised from light petroleum (b. p. 40—60°) or acetone at −50°, in needles, m. p. 34—35° (Found : C, 71·2; H, 7·3. $C_{13}H_{16}O_3$ requires C, 70·9; H, 7·3%).

(c) *Monobromo-ketones from* sec.-*Butyl Methyl Ketone.*—The monobromo-ketones from *sec.*-butyl methyl ketone (b. p. 50—108°/30 mm.; 142 g.) on fractionation at 35 mm. (reflux ratio 20 : 1) gave the following fractions (total 112 g.) : (i) b. p. up to 64·0° (3·0 g.); (ii) b. p. 64·0—69·0° (3·1 g.); (iii) b. p. 69·0—71·0° (9·0 g.); (iv) b. p. 71·5—72·5° (50·8 g.); (v) b. p. 72·5—73·0° (2·1 g.); (vi) b. p. 73·0—74·5° (2·4 g.); (vii) b. p. 74·5—81·0° (4·7 g.); (viii) b. p. 81·0—85·0° (2·6 g.); (ix) b. p. 85·0—89·0° (3·3 g.); (x) b. p. 89·0—91·0° (4·4 g.); (xi) b. p. 91·0—93·0° (4·4 g.); (xii) b. p. 93·0—95·0° (5·6 g.); (xiii) b. p. 95·0—100·0° (3·2 g.); (xiv) column stripped at 18 mm. (13·7 g.).

Fractions (iv) on redistillation gave 3-*bromo*-3-*methylpentan*-2-*one* (48·6 g.), b. p. 65°/24 mm., 69°/30 mm., 74°/39 mm., 80·5°/50 mm., n_D^{21} 1·4634 (Found : Br, 45·1. $C_6H_{11}OBr$ requires Br, 44·6%). 3-*Anilino*-3-*methylpentan*-2-*one* had b. p. 125°/1·5 mm., n_D^{20} 1·5476 (Found : C, 75·1; H, 9·1. $C_{12}H_{17}ON$ requires C, 75·5; H, 9·0%).

Fractions (v)—(xiii) were refluxed for 3 hours with thiourea (22·4 g.), ethanol (50 c.c.), and water (50 c.c.). To the cooled product 2*N*-aqueous sodium hydroxide (150 c.c.) was added and the mixture was refluxed for ½ hour. The mixture was cooled, acidified with dilute hydrochloric acid, and extracted several times with ether. The evil-smelling ethereal layer on distillation gave impure 3-mercapto-3-methylpentan-2-one, b. p. 75—77°/27 mm., n_D^{20} 1·4602 (2·6 g.) (Found : C, 57·1; H, 9·8. $C_6H_{12}OS$ requires C, 54·1; H, 9·2%). This compound was so offensive that further purification was not attempted. Pure 3-bromo-3-methylpentan-2-one on similar treatment with thiourea gave 5·1 g. of the mercapto-ketone (38·6%). The low yield indicated that this method was not suitable for quantitative determination of tertiary bromo-ketones; but, on the assumption that the yield was the same in the two experiments, fraction (v)—(xiii) contained about 9·0 g. of 3-bromo-3-methylpentan-2-one making the total quantity of this bromo-ketone 65—66 g. (56—57 g. estimated from the graph). The aqueous layer was then made alkaline with aqueous sodium hydroxide, and extracted with ether. Distillation of the ethereal solution gave 2-*amino*-4-*sec.*-*butylthiazole* as a colourless viscous liquid, b. p. 109—111°/2 mm., n_D^{21} 1·5558 (13·8 g.) (Found : C, 53·7; H, 7·9. $C_7H_{12}N_2S$ requires C, 53·8; H, 7·7%). The *picrate* crystallised from acetone in yellow needles, m. p. 183—185° (decomp.) (Found : C, 40·3; H, 3·7. $C_7H_{12}N_2S,C_6H_3N_3O_7$ requires C, 40·5; H, 3·9%). The thiazole prepared from pure 1-bromo-3-methylpentan-2-one (diazomethane method) had b. p. 110°/1·5 mm., n_D^{20} 1·5562; the picrate had m. p. 188—189° (decomp.). A mixture with the picrate from the crude ketones showed no depression.

On the assumption that the yield of the thiazole from the bromomethyl ketone was 93—97% (as in similar cases), these fractions must have contained 16—17 g. of 1-bromo-3-methylpentan-2-one. Interpolation from the graph of weight of distillate against boiling point gave 18—19 g., whilst direct determination by application of the thiazole method to a portion of the crude ketones before distillation gave 18·7 g.

(d) *Monobromo-ketones from Methyl* iso*Propyl Ketone.*—The monobromo-ketones from this ketone (b. p. 75—120°/150 mm.; 172 g.) on fractionation at 150 mm. (reflux ratio 20 : 1) gave the following fractions (total 130 g.) : (i) b. p. to 80·0° (2·8 g.); (ii) b. p. 80·0—83·0° (1·4 g.); (iii) b. p. 83·0—83·5° (38·6 g.); (iv) b. p. 83·5—84·0° (38·8 g.); (v) b. p. 84·0—90·0° (3·4 g.); (vi) b. p. 90·0—96·0° (1·9 g.); (vii) b. p. 96·0—103·0° (3·7 g.); (viii) b. p. 103·0—105·5° (24·5 g.); (ix) b. p. 105·5—119·0° (5·7 g.); (x) column stripped at 60 mm. (9·4 g.).

Fractions (iii) and (iv) on redistillation gave 3-bromo-3-methylbutan-2-one (70 g.), b. p. 78°/100 mm., 82°/120 mm., 86°/140 mm., 90°/150 mm., 93·5°/160 mm., n_D^{20} 1·4575 (Catch *et al.* give b. p. 83—84°/150 mm., n_D^{16} 1·4590) (Found : Br, 47·4. Calc. for C_5H_9OBr : Br, 48·5%).

Fraction (viii) on redistillation gave 1-bromo-3-methylbutan-2-one (20 g.), b. p. 93°/75 mm., 101°/100 mm., 107—108°/125 mm., n_D^{20} 1·4635 (Found : Br, 46·7. Calc. for C_5H_9OBr : Br, 48·5%). The low bromine analysis indicated that this sample certainly contained very little dibromo-ketone. Catch *et al.* (*loc. cit.*) record b. p. 86°/50 mm., $n_D^{14\cdot5}$ 1·4467.

A sample of the above bromomethyl ketone on treatment with thiourea gave 2-*amino*-4-iso*propylthiazole*, a pale yellow oil, b. p. 105—107°/0·5 mm., n_D^{20} 1·5616 (Found : C, 50·4; H, 7·2. $C_6H_{10}N_2S$

requires C, 50·7; H, 7·1%). The *picrate* crystallised from acetone in yellow needles, m. p. 209—211° (decomp.) (Found: C, 39·6; H, 3·7; N, 19·2. $C_8H_{15}N_3S,C_6H_3O_7N_3$ requires C, 38·8; H, 3·5; N, 18·9%).

50 G. of the stripped brominated mixture were treated with thiourea and worked up as already described for the bromination product of *sec.*-butyl methyl ketone (p. 2440). The yield of 2-amino-4-*iso*propylthiazole was 10·0 g., which is equivalent to 24% of 1-bromo-3-methylbutan-2-one in the mixture (the reaction being assumed to afford a 97% yield). This figure agrees well with that obtained by interpolation from the graph (*vis.*, 25%).

(e) *Monobromo-ketones from* n-*Amyl Methyl Ketone.*—The monobromo-ketones (b. p. 50—89°/7 mm.; 535 g.) were fractionated at 4 mm. through a 160-cm. column packed with Fenske helices. The following fractions (total 491 g.) were collected: (i) b. p. 25—54° (6·5 g.); (ii) b. p. 54—61·5° (8·9 g.); (iii) 61·5—64° (246·8 g.); (iv) 64—67·5° (6·5 g.); (v) 67·5—71° (4·5 g.); (vi) 71—74° (5·6 g.); (vii) 74—77·5° (6·3 g.); (viii) 77·5—78·5° (117·9 g.); (ix) 78·5—79·0° (48·3 g.); (x) 79·0° (30·9 g.); (xi) 79—85° (9·3 g.).

Fraction (iii) consisted of 3-*bromoheptan*-2-*one*, n_D^{18} 1·4610 (Found: C, 43·8; H, 7·1; Br, 41·5. $C_7H_{13}OBr$ requires C, 43·5; H, 6·7; Br, 41·5%). The small forerun indicated that the preliminary stripping from unchanged ketone was too thorough. The stripping (82 g.) was therefore analysed (Found: Br, 22·39, 22·05%). This fraction therefore contained up to 44 g. of monobromo-ketone, largely 3-bromoheptan-2-one, making the total quantity of this ketone, 290 g. A sample of this bromo-ketone on treatment with thiourea gave 2-*amino*-5-n-*butyl*-4-*methylthiazole*, colourless needles, m. p. 41—42°, from light petroleum (b. p. 40—60°) (Found: C, 56·2; H, 8·0. $C_8H_{14}N_2S$ requires C, 56·5; H, 8·2%). The *picrate* crystallised from acetone in yellow needles, m. p. 192—193° (decomp.) (Found: C, 43·3; H, 4·7. $C_8H_{14}N_2S,C_6H_3O_7N_3$ requires C, 42·1; H, 4·3%). 3-*Anilinoheptan*-2-*one*, a yellow oil, had b. p. 132°/1·0 mm., n_D^{20} 1·5449 (Found: C, 77·2; H, 9·2. $C_{13}H_{19}ON$ requires C, 76·1; H, 9·3%).

Fractions (viii)—(x) consisted of 1-bromoheptan-2-one, n_D^{18} 1·4650 (Found: C, 43·8; H, 7·1; Br, 41·3. Calc. for $C_7H_{13}OBr$: C, 43·5; H, 6·7; Br, 41·5%). Catch *et al.* record b. p. 96°/14 mm., n_D^{18} 1·4645. A sample of this bromo-ketone on treatment with thiourea gave 2-*amino*-4-n-*amylthiazole*, a pale yellow oil, b. p. 134°/1·5 mm., n_D^{20} 1·5429 (Found: C, 56·2; H, 8·1; N, 15·9. $C_8H_{14}N_2S$ requires C, 56·5; H, 8·2; N, 16·5%). The *picrate* crystallized from acetone in yellow needles, m. p. 183—184° (decomp.) (Found: C, 42·5; H, 4·0; N, 17·9. $C_8H_{14}N_2S,C_6H_3O_7N_3$ requires C, 42·1; H, 4·3; N, 17·5%). 1-*Anilinoheptan*-2-*one* crystallized from light petroleum (b. p. 60—80°) in colourless elongated plates, m. p. 73—74° (Found: C, 76·0; H, 9·3; N, 7·0. $C_{13}H_{19}ON$ requires C, 76·1; H, 9·2; N, 6·8%).

(f) *Monobromo-ketones from* n-*Hexyl Methyl Ketone.*—The monobromo-ketones (506 g.; b. p. 56—93°/3 mm.) were fractionated at 2—3 mm. through a 200-cm. column packed with Fenske helices. The following fractions (total 477 g.) were collected: (i) b. p. 32·0° (72·0 g.); (ii) b. p. 32·0—61·0° (9·3 g.); (iii) b. p. 61·0—61·5° (3·9 g.); (iv) b. p. 61·5—63·5° (299·9 g.); (v) b. p. 63·5—67·5° (13·0 g.); (vi) b. p. 67·5—71·5° (1·9 g.); (vii) b. p. 71·5—76·0° (4·4 g.); (viii) b. p. 76·0—77·4° (4·4 g.); (ix) b. p. 77·4—79·0° (53·7 g.); (x) b. p. 79·0—84·0° (18·8 g.).

Fraction (i) consisted of unchanged *n*-hexyl methyl ketone, n_D^{20} 1·4150. Fraction (iv) consisted of 3-*bromo-octan*-2-*one*, n_D^{20} 1·4615 (Found: Br, 37·6. $C_8H_{15}OBr$ requires Br, 38·6%). On treatment with thiourea it gave 2-*amino*-5-n-*amyl*-4-*methylthiazole*, a colourless, waxy solid, m. p. 26—27° (from light petroleum, b. p. 40—60°, at a low temperature) (Found: C, 59·1; H, 8·8; N, 15·3; S, 17·2. $C_9H_{16}N_2S$ requires C, 58·7; H, 8·7; N, 15·2; S, 17·4%).

Fractions (viii)—(x) consisted largely of 1-*bromo-octan*-2-*one*. The preliminary stripping from dibromo-ketones had evidently been a little too efficient, and the proportion of the bromomethyl ketone was almost certainly underestimated. Fraction (ix) had n_D^{19} 1·4660 (Found: C, 46·2; H, 7·5; Br, 40·2. $C_8H_{15}OBr$ requires C, 46·3; H, 7·2; Br, 38·6%). 1-*Anilino-octan*-2-*one* crystallized from light petroleum (b. p. 60—80°) in colourless elongated plates, m. p. 67—68° (Found: N, 6·2. $C_{14}H_{21}ON$ requires N, 6·4%).

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538. *Elimination Reactions. Part II.* Base-catalysed Ionisation and Substitution Reactions of Ketones and Nitroparaffins.*

By H. M. E. Cardwell.

Two opposing rules of orientations of base-catalysed substitution reactions of ketones are observed. These rules correspond to the Hofmann and the Saytzeff type of control of the preliminary elimination reaction. It is shown, by analysis of kinetic data from the nitroparaffins, that these rules may be due to the fact that alkyl groups inductively hinder the loss of a proton to a base, but that once this has occurred they stabilise the resulting ion in a resonant or hyperconjugative manner.

Hughes (*Nature*, 1941, **147**, 813) has pointed out that the rates of base-catalysed iodination of acylbenzenes (Table I) as determined by Evans and Gordon (*J.*, 1938, **1434**) indicate that alkyl groups act in an inductive manner and hinder the loss of a proton to a base. The large decrease in rate of loss of a proton caused by replacement of one hydrogen atom by Me, the slight decrease in rate on replacing Me by Et, and the further sharp decrease in rate on replacing the second hydrogen atom by Me are entirely and only consistent with this explanation.†

Table I.

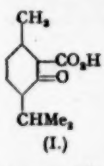
Rates of AcO^-*-catalysed iodination of* $Ph\cdot CO\cdot CHR_1R_2$.

R_1, R_2 =	H, H	H, Me	H, Et	Me, Me
Rate	238	37	29	7

(The rate for $Ph\cdot CO\cdot CH_3$ has been adjusted to the rate of ionisation of $CH_3\cdot NO_2$ so that the figures are directly comparable with those given in Table II.)

Tautomerism in ketones is therefore very similar to elimination reactions of sulphonium salts; under basic conditions the Hofmann rule is operative in ionisation of ketones and bimolecular elimination reactions of sulphonium salts; but on crossing the mechanistic border to acid-catalysed enolisation of ketones and to unimolecular elimination reactions of sulphonium salts Saytzeff's rule becomes operative.

There are a number of base-catalysed substitution reactions of ketones which follow Hofmann's rule of orientation of substitution. For instance, excellent yields of carboxylic acids ($Alk\cdot CH_2\cdot CO_2H$ from $\cdot CO\cdot CH_3$ *via* $\cdot CO\cdot CHal_2$) are obtained on base-catalysed iodination of alkyl methyl ketones (Simonsen, *J.*, 1922, **121**, 2292; Semmler and Schiller, *Ber.*, 1927, **60**, 1591). Many base-catalysed aldol condensations conform to this type of orientation; for instance, aromatic aldehydes condense preferentially on the CH_3 group of *n*-alkyl methyl ketones (Harries and Warunis, *Annalen*, 1903, **330**, 257; Warunis and Lekos, *Ber.*, 1910, **43**, 654; Zafiriadis, *Compt. rend.*, 1950, **230**, 452). The aldol condensation is, however, a reversible reaction, and the orientation of condensation is sensitive to the experimental conditions used. Carboxylation also follows Hofmann's rule. In this reaction the ketone is converted rapidly and irreversibly into the sodium enolate by treatment with a slight excess of sodium triphenylmethide in ether and is then treated with carbon dioxide. The latter reaction also appears to be very rapid. Using this technique, Levine and Hauser (*J. Amer. Chem. Soc.*, 1944, **66**, 1768) found that the product was solely $CH_3R\cdot CO\cdot CH_2\cdot CO_2H$ from the ketones $CH_3R\cdot CO\cdot CH_3$ (R = Me, Et, *n*-C_5H_{11}, and Pr^i). With sodamide in ether the enolate does not appear to be produced immediately and irreversibly, for Gardner, Perkin, and Watson (*J.*, 1910, **97**, 1756) found that (−)-menthone and (+)-*iso*menthone both gave a carboxy-(+)-*iso*menthone (I), indicating that inversion had occurred [with (−)-menthone] at the $\cdot CHPr^i\cdot$ asymmetric centre (see note added in proof, p. **2444**).



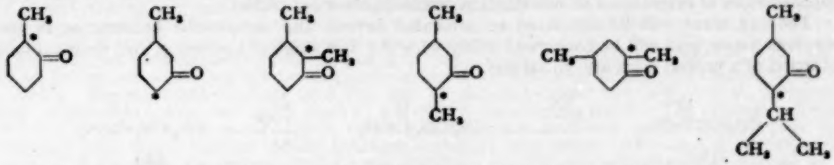
(I.)

With other substituting reagents (alkyl halides, alkyl sulphates, diethylaminobutanone methiodide) substitution occurs on the most substituted carbon atom. For instance, with the following cyclic ketones substitution occurs on the carbon atom marked * (Cornubert, Humeau,

* Part I, preceding paper.

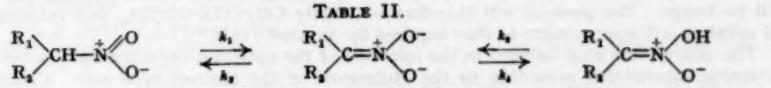
† This paper is restricted, as is the previous paper, to saturated acyclic or monocyclic ketones containing no hetero-substituent other than the single carbonyl oxygen atom. The use of rate data from the acetophenones therefore presents an anomaly which it is hoped to resolve in a later paper.

Bihan, and Maurel, *Bull. Soc. chim.*, 1931, **49**, 1260). This is substitution in accordance with the extended Saytzeff rule given in the preceding paper.



A clue to the origin of these opposed rules of orientation of substitution is given by the observation that the reactions following Hofmann's rule are rapid, whilst those following Saytzeff's rule are slow, but the most convincing evidence comes from kinetic data (Table II) on rates of ionisation and equilibrium concentrations of ions of the nitroparaffins (Maron and La Mer, *J. Amer. Chem. Soc.*, 1938, **60**, 2588; Turnbull and Maron, *ibid.*, 1943, **65**, 212; Bell and Swarzenbach, private communication).

TABLE II.



Serial No.	R_1, R_2 = H, H (i).	Me, H (ii).	Et, H (iii).	Me, Me (iv).
1	k_1	238	39	29	2·1
2	k_1/k_2	$6·2 \times 10^{-11}$	$3·5 \times 10^{-9}$	$1·1 \times 10^{-9}$	$2·1 \times 10^{-9}$
3	$10^{-9}k_2$	3840	112	263	1
4	$10^9k_3/k_4$	600	39	15	7·7

The variations in rates of ionisation (serial No. 1) of the nitroparaffins and ketones (Table I) on change of the alkyl substituents are almost identical; the inductive effect of alkyl substituents is therefore operative to the same extent in both reactions. The figures for the equilibrium constants (serial No. 2), however, reveal the paradox that 2-nitropropane (iv), which of the series most slowly loses a proton, is the strongest acid. The figures in serial No. 3 resolve this paradox, for they show that the extra Me in $CHMe{:}N\overset{+}{<}\overset{O^-}{O}$ stabilises this ion relative to $CH_2{:}N\overset{+}{<}\overset{O^-}{O^-}$, and that Et is less effective than Me as a stabiliser. This clearly is a resonance or hyperconjugative effect, for the normal order of this effect (2Me > Me + H > Et + H > 2H) is observed. Alkyl substituents will stabilise the two systems $>C{:}N\overset{+}{<}\overset{O^-}{O^-}$ and $>C{:}N\overset{+}{<}\overset{OH}{O^-}$ in an equivalent manner; hence it is not surprising that their effect on k_2/k_4 (serial No. 4) is largely inductive. The effect of alkyl groups on the rates of addition of a proton to carbon in the mesomeric ion may be expressed in a slightly different manner. In the mesomeric

system (A) $\rightleftharpoons$ (B), it is usually assumed that (B) makes a much larger contribution than (A), but it is quite reasonable to assume that variations in the substituents R_1 and R_2 will vary the electron distribution in the mesomeric ion, and this variation may be expressed by saying that the relative contributions made by (A) and (B) are varied. The larger the contribution made by (A) the greater will be the rate of addition of a proton to carbon. Replacement of a hydrogen atom by Et will increase the relative contribution made by (B), an effect that will be accentuated by replacement of Et by Me. The above system is analogous to the three carbon system:

and the rate data for k_2 (cols. i—iii) may be compared with the proportions of αβ- (addition of H^+ to A) and βγ- (addition of H^+ to B) unsaturated acids obtained on equilibration of these systems. The percentages of αβ-unsaturated compound were: R = H, 100; Me, 5; Et, 33 (Kon and May, *J.*, 1927, 1549; Hughes *loc. cit.*; de la Mare, Hughes, and Ingold, *J.*, 1948, 17).

With ketones, experimental difficulties have so far precluded the measurements of rates of ionisation and equilibrium concentrations of enolate ions. The assumption that alkyl groups

act qualitatively in the same manner in base-catalysed reactions of nitroparaffins and ketones is, however, strengthened by the observation that it provides a satisfactory explanation of the opposed rules of orientation of substitution reactions observed earlier.

The argument will be restricted to saturated acyclic and monocyclic ketones, as in the previous paper, and will be concerned solely with the first stage of ionisation, *i.e.*, removal and addition of a proton from and to carbon.

$$\underset{\text{(A.)}}{CH_3{\cdot}CH{:}\overset{O^-}{\overset{|}{C}}{\cdot}CH_3} \underset{k_2^a}{\overset{k_1^a}{\rightleftharpoons}} CH_3{\cdot}CH_2{\cdot}\overset{O}{\overset{\|}{C}}{\cdot}CH_3 \underset{k_2^b}{\overset{k_1^b}{\rightleftharpoons}} \underset{\text{(B.)}}{CH_3{\cdot}CH_2{\cdot}\overset{O^-}{\overset{|}{C}}{:}CH_2}$$

In the particular case of ethyl methyl ketone, by analogy with the nitroparaffins, k_1^b will be greater than k_1^a, but k_2^b will be much greater than k_2^a. If, therefore, a substituting reagent is used which reacts with the enolate ion as soon as it is formed, the product will be largely $CH_3{\cdot}CH_2{\cdot}CO{\cdot}CH_2X$ ($k_1^b > k_1^a$) (Hofmann-type rule). If, however, conditions are used which allow equilibration of the enolate ions before substitution takes place, the orientation may be controlled by the ratio of k_1^a/k_2^a to k_1^b/k_2^b, and by analogy with the nitro-paraffins the former will be larger. The product will therefore be largely $CH_3{\cdot}CHX{\cdot}CO{\cdot}CH_3$, and orientation of substitution will approximate to that required by Saytzeff's rule.*

The above ideas may be used in the selection of the optimum experimental conditions for obtaining substitution according to the Hofmann- or the Saytzeff-type rule. Clearly, the cationoid reactivity of the attacking reagent is of supreme importance; where it is highly reactive, and where the substitution is irreversible, orientation will follow the Hofmann rule regardless of the experimental conditions used.

In these cases it is often possible to use a weak base in a solvent which can provide the proton necessary for equilibration of the enolate ions, but if the ion is attacked as soon as it is formed, the rate of formation of the ions will control orientation of substitution.

With alkyl halides and sulphates it is necessary to convert the ketone completely into the enolate ion (to minimise aldol type condensation) before substitution takes place, as the unchanged ketone is a more reactive cationoid reagent than the halides or sulphates. These conditions do not, in general, favour equilibration, for they involve the use of strong bases (sodium triphenylmethide, sodamide, or sodium *tert.*-alkoxides) in aprotic solvents; but once a small amount of the ketone has been alkylated, the resulting alkyl ketone can act as a proton source and allow equilibration of the small amount of enolate ion in solution. With the more reactive alkylating agents (*e.g.*, methyl sulphate) it should prove possible to increase the cationoid reactivity sufficiently, by use of a solvent of high dielectric constant, for orientation of substitution to follow the Hofmann-type rule. Clearly, the new solvent must not be one which increases significantly the possibility of equilibration of the ions or of self-condensation of the ketone. The only solvents which satisfy these conditions are the highly basic solvents such as liquid ammonia. In conformity with these ideas, but quite independently, Dr. Friedmann in these laboratories has recently discovered that methyl sulphate reacts rapidly with the sodium enolate of 2-methyl*cyclo*hexanone in liquid ammonia at −70° to give 80% of 2 : 6- and only 20% of 2 : 2-dimethyl*cyclo*hexanone. This is almost a complete inversion of the orientation found in the slow alkylation with this reagent in ether.

An adequate test of these ideas would clearly involve much work, but they are put forward now in the hope that they will facilitate the choice of the optimum experimental conditions for the preparation of particular substituted ketones.

[*Added in proof, July 5th*, 1951.] The preparation of (−)-2-carbomethoxymenthone by successive treatment of (−)-menthone with ethereal sodium triphenylmethide, carbon dioxide, and ethereal diazomethane has shown that these reactions are rapid and largely irreversible under the conditions used. Analysis of the mixture of keto-esters prepared in this way from an unsymmetrical ketone therefore gives a rough measure of the relative rates of loss of a proton from the two reactive positions.

* The more stable ion of any pair should be the least reactive not only to a proton but to most cationoid reagents. Hence, change over to Saytzeff-type orientation of substitution should not occur at once on passing from zero to first-order reactions with respect to the cationoid substituting reagent. A correlation between rules of substitution and cationoid reactivity of the reagents would be explicable in terms of probability factors and activation energies (Birch, Faraday Soc. Discussion "The Labile Molecule," 1947, p. 247, 262; Dewar, *ibid.*, p. 261; "Electronic Theory of Organic Chemistry," Oxford Univ. Press, 1949, p. 103) but it would not be easy to deduce in this manner that substitution with unreactive cationoid reagents would approximate to the Saytzeff-type rule.

(−)-2-*Carbomethoxymenthone*.—(−)-Menthone (5 g.; $[\alpha]_D^{20}$ −22·9°) in dry ether (20 c.c.) was treated with slightly less than one equiv. of ethereal sodium triphenylmethide. The ethereal suspension of the sodium enolate was immediately run on excess of chopped solid carbon dioxide. When evolution of carbon dioxide had ceased the mixture was twice extracted with ice-cold water. The combined aqueous extracts were extracted five times with ether, and were acidified with ice-cold 2N-sulphuric acid. The liberated (−)-2-carboxymenthone was extracted with ether. The ethereal extract was rapidly clarified with magnesium sulphate and then (95 c.c.) gave a rotation of −2·21° in a 3-dm. tube (from the amount of methyl ester subsequently obtained the acid must have had a minimum $[\alpha]_D$ of −24·1°). The menthone obtained from this acid by decarboxylation had $[\alpha]_D^{20}$ −20·4°, indicating that negligible reversion had taken place at the ·CHPr^i· asymmetric centre. The ethereal solution (90 c.c.) of the acid was esterified with ethereal diazomethane and then washed rapidly with 2N-sulphuric acid, water, aqueous sodium hydrogen carbonate, and water. After drying ($MgSO_4$) and removal of the ether the residue (2·92 g.) was distilled. (−)-2-*Carbomethoxymenthone* distilled as a colourless oil with a sweet menthone-like odour, b.p. 122—124°/6—7 mm., $[\alpha]_D^{20}$ −29·0° (*c*, 6 in methanol) (Found: C, 67·3; H, 9·5. $C_{12}H_{20}O_3$ requires C, 67·9; H, 9·4%). The ester gave a deep green colour with methanolic ferric chloride. On addition of a few drops of concentrated sulphuric acid to a solution of the ester (628·6 mg.) in methanol (10 c.c.) the rotation (1-dm. tube) changed from −1·82° to +0·37° in 6 days but mutarotation was not complete when this note was submitted.

The author thanks Mr. R. P. Bell and Dr. C. A. Friedmann for permission to quote unpublished work, and for many stimulating discussions. This work was completed during tenure of an Imperial Chemical Industries Fellowship.

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[*Received, March 30th*, 1951.]

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539. *Experiments on the Synthesis of the Pyrethrins. Part VI.* New Syntheses of the Cinerolones.*

By L. CROMBIE, S. H. HARPER, R. E. STEDMAN, and D. THOMPSON.

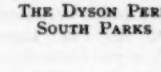
The fourteen-stage synthesis of (±)-*cis*-cinerolone (Part IV), involving *n*-pent-3-yn-1-ol as the key intermediate, is shortened to ten stages and improved in overall yield by the incorporation of a new sequence of reactions.

A superior ten-stage synthesis of (±)-*cis*-cinerolone is described, involving *n*-but-2-yn-1-ol as the key intermediate.

Synthetic (±)-*cis*-cinerolone is compared with naturally derived (±)-*cis*-cinerolone by means of their infra-red absorption spectra. It is shown that the latter is contaminated with (±)-pyrethrolone.

A homologue of *cis*-cinerolone, (±)-*cis*-*n*-pent-2-enylrethrolone is prepared and esterified with (+)-*trans*-chrysanthemic acid. The resultant ester is comparable with allethrin and the natural pyrethrins in toxicity towards houseflies.

IN Part IV two of us (Crombie and Harper, *J.*, 1950, 1152) described a fourteen-stage synthesis in 0·2% overall yield of (±)-*cis*-cinerolone (I*a*; "natural cinerolone"), following our route *B* (cf. Part V *). The essential feature of route *B* is a five-step process for proceeding



(I.)

(I*a*; R = CH_2·CH:CHMe-*cis*)
(I*b*; R = CH_2·CH:CHMe-*trans*)
(I*c*; R = CH_2·CH:CHEt-*cis*)

from R·CH_2·OH to R·CH_2·CO·CH_2·CO_2Me (where R is the alkenyl group ultimately becoming the side-chain of I). We now find that the following three-stage scheme (hereinafter referred to as route *E*), of which the second stage is based on the work of Newman and Booth (*J. Amer. Chem. Soc.*, 1945, **67**, 154), gives a better overall yield:

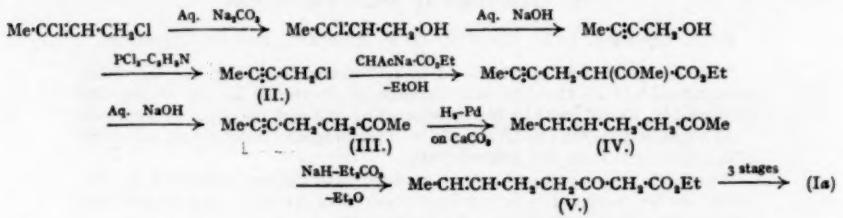
$$\text{R·CH}_2\text{·OH} \xrightarrow{\text{PBr}_3\text{–C}_5\text{H}_5\text{N}} \text{R·CH}_2\text{Br} \xrightarrow[\text{Ac}_2\text{O at } -75^\circ]{\text{Mg–Et}_2\text{O, then}} \text{R·CH}_2\text{·COMe} \xrightarrow[-\text{Et}_2\text{O}]{\text{NaH–Et}_2\text{CO}_3} \text{R·CH}_2\text{·CO·CH}_2\text{·CO}_2\text{Et}$$

The bromide from the more accessible *trans*-*n*-pent-3-en-1-ol was converted into *trans*-*n*-hept-5-en-2-one (*trans*-crotylacetone) (60% yield), a ketone we had obtained earlier by the use of our route *D* (Part V *), and thence through ethyl *trans*-2-keto-*n*-hept-5-ene-1-carboxylate into (±)-*trans*-cinerolone (I*b*).

* Part V, *J.*, 1950, 3552.

In the original synthesis semi-hydrogenation of *n*-pent-3-yn-1-ol to *cis*-*n*-pent-3-en-1-ol was used as the means of introducing the *cis*-crotyl side-chain. The acetylenic alcohol was prepared by a bromination–dehydrobromination treatment of the mixed *cis*- and *trans*-*n*-pent-3-en-1-ols, themselves obtained through ring scission of the mixed *cis*- and *trans*-3-chloro-2-methyltetrahydrofurans by sodium in ether (Crombie and Harper, *J.*, 1950, 873, 1715), but the yield over the bromination–dehydrobromination stages was poor (33%; 27% overall). We have sought, therefore, a more satisfactory preparation of *n*-pent-3-yn-1-ol. Miss J. Sandiford (Harper and Sandiford, unpublished observations) has found that the ring scission of the mixed *cis*- and *trans*-3-chloro-2-methyltetrahydrofurans by sodamide in liquid ammonia yields *n*-pent-3-yn-1-ol directly, albeit in only 28% yield. According to an abstract (*Bull. Soc. chim.*, 1909, [iv], 6, 98) Iotsitch prepared this alcohol in 95% yield by interaction of a prop-1-ynylmagnesium halide with ethylene oxide, but this is not described in Beilstein nor has the use of this method since been reported. We have found that reaction of the more accessible sodiopropyne in liquid ammonia with ethylene oxide gives a negligible yield. The use of route *E*, together with the shorter preparation of *n*-pent-3-yn-1-ol, provides a ten-stage synthesis of (±)-*cis*-cinerolone in about twice the overall yield of the original synthesis, but another route, described below, gives still better results.

In Part IV we envisaged an alternative synthesis of (±)-*cis*-cinerolone, by route *D*, requiring *n*-but-2-yn-1-ol as the key intermediate. Hitherto, this has been accessible only from propyne and paraformaldehyde in low yield (16%) [Yvon, *Compt. rend.*, 1925, 180, 748; Hurd and Cohen, *J. Amer. Chem. Soc.*, 1931, 53, 1068; though Iotsitch (*loc. cit.*) is stated to have obtained an 80% yield], but very recently Hatch and Nesbitt (*J. Amer. Chem. Soc.*, 1950, 72, 727) have described an excellent preparation, from commercially available 1 : 3-dichlorobut-2-ene. Exploiting this we have investigated the following ten-stage route to (±)-*cis*-cinerolone:

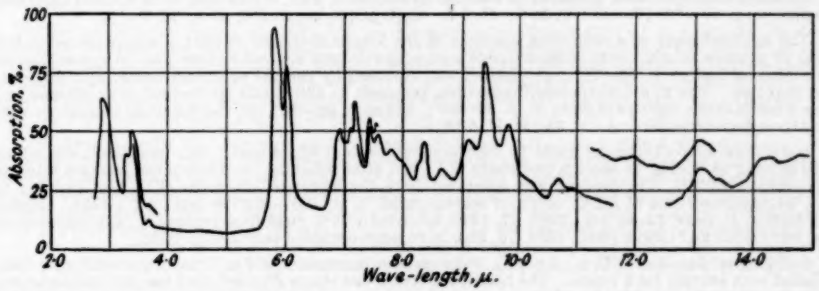


Conversion of *n*-but-2-yn-1-ol into the chloride gave a product of boiling point 102°, whereas Hurd and Cohen (*loc. cit.*) reported 81—84°. We observed, however, the distillation of a little water azeotrope at this lower temperature and these authors' product may well have been this azeotrope [this view has since been confirmed by Hatch and Chiola (*ibid.*, 1951, 73, 360)]. Alkylation of ethyl sodioacetoacetate with this chloride (II) followed by ketonic fission gave *n*-hept-5-yn-2-one (III). Contrary to our usual experience of this reaction the neutral oil, removed before acidification and warming to liberate the ketone, contained *n*-hept-5-yn-2-one equivalent to a further 8% yield—formed by decarboxylation in alkaline solution. Semi-hydrogenation to *cis*-*n*-hept-5-en-2-one (IV; *cis*-crotylacetone) was carried out at this stage, as elimination of saturated or acetylenic ketonic contaminants by distillation was considered to be easier here than subsequently. This product was almost free from *trans*-isomer, for its infra-red spectrum showed only a slight hump at 10·3 μ., whereas the spectra of the specimens of *trans*-crotylacetone prepared by routes *D* and *E* both exhibited a strong band at this wave-length.

Difficulty was experienced in characterising *cis*- and *trans*-crotylacetones. The 2 : 4-dinitrophenylhydrazones (*cis*-, m. p. 83·0—84·5°; *trans*-, m. p. 69—70°) proved more satisfactory than the semicarbazones (*cis*-, m. p. 124—125°), the derivatives of the *cis*-isomer being obtained more readily than those of the *trans*-isomer. Although the specimens of *trans*-crotylacetone prepared by routes *D* and *E* both gave the same 2 : 4-dinitrophenylhydrazone, a semicarbazone (m. p. 69—70°) could be isolated only from the former specimen. In contrast, von Braun and Gossel (*Ber.*, 1924, 57, 373) recorded m. p. 97° for the semicarbazone of *trans*-crotylacetone prepared by the acetoacetic ester method, and Kimel and Cope (*J. Amer. Chem. Soc.*, 1943, 65, 1992) reported m. p. 104·5—105·5° for the semicarbazone of a crotylacetone obtained by thermal rearrangement of 1-methylallyl acetoacetate. The latter may have been impure *cis*-crotylacetone, but it seems likely that *trans*-crotylacetone has not been obtained quite pure; certainly

the infra-red spectra of both of our preparations showed indications of the presence of contaminants. Nevertheless it is of interest that the *cis*-derivatives melt higher than those of *trans*-crotylacetone. Carbethoxylation of *cis*-*n*-hept-5-en-2-one (IV) gave ethyl 2-keto-*cis*-*n*-hept-5-ene-1-carboxylate (V), which was converted into (±)-*cis*-cinerolone in 1% overall yield by the same three stages of hydrolysis, condensation with pyruvaldehyde, and cyclisation as were used previously (Part IV, *loc. cit.*).

Comparison of the infra-red spectrum of the synthetic (see figure) with the recently published spectrum of naturally derived (±)-*cis*-cinerolone (Cupples, *J. Amer. Chem. Soc.*, 1950, **72**, 4522) shows excellent agreement: the peaks in the two spectra can be matched with only one significant exception, namely, a small peak at 11·1 μ. for the latter compound. The most likely impurity in the naturally derived material would be (±)-pyrethrolone and we have previously remarked (Part IV, *loc. cit.*) that ozonolysis, ultra-violet light absorption, and refractive index indicate that this is present in the specimens of naturally derived (±)-*cis*-cinerolone so far obtained. Examination of the infra-red spectrum of naturally derived (±)-pyrethrolone (B-2) (Crombie and Harper, unpublished observations) shows that it has a fairly strong absorption band at 11·1 μ. and comparison with the spectrum of naturally derived (±)-*cis*-cinerolone indicates that it is just this band which would be most clearly evident if pyrethrolone were present as an impurity. The spectrum of synthetic (±)-*cis*-cinerolone also shows the absence of anything more than traces of *trans*-isomer. The spectrum of (±)-*trans*-cinerolone (Cupples, *loc. cit.*) has a strong band at *ca.* 10·3 μ. in common with other *trans*-compounds of the type R·CH=CH·R′ (cf. Crombie and Harper, *J.*, 1950, 873; and references



cited therein). The wave-lengths (μ.) and assignments of the more important absorption bands of synthetic and naturally derived (±)-*cis*-cinerolone are listed in the Table.

(±)-*cis*-Cinerolone.	Bonded OH	Unsat. aliph. C—H	Satd. CH_2, CH_3, and C—H	C=O	C=C	Satd. CH_2	C—CH_3
Synthetic [1]	2·92	3·31	3·43	5·88	6·06	6·96	7·22
Naturally derived [2]	2·91	3·30	3·42	5·89	6·06	6·96	7·23

[1] This work. [2] Cupples, *loc. cit.*

We have also prepared a true homologue of *cis*-cinerolone, (±)-*cis*-*n*-pent-2-enylrethrolone (I*c*) (for an exposition of this nomenclature, see Harper, *Chem. and Ind.*, 1949, 636) by route *C* (cf. Part V, *loc. cit.*). The starting material was "leaf alcohol," isolated from the tailings of Brazilian peppermint oil and shown by Crombie and Harper (*loc. cit.*) to be stereochemically pure *cis*-*n*-hex-3-en-1-ol. This was converted successively through the bromide and cyanide into *cis*-*n*-hept-4-enoic acid following Treff and Werner (*Ber.*, 1935, **68**, 640) and Hunsdiecker (*Ber.*, 1942, **75**, 460), though the modified procedure of LaForge, Green, and Gersdorff (*J. Amer. Chem. Soc.*, 1948, **70**, 3707) was used for the second stage. *cis*-*n*-Hept-4-enoyl chloride was converted into methyl *cis*-2-keto-*n*-oct-5-ene-1-carboxylate by *C*-acylation of acetoacetic ester (*loc. cit.*; cf. Part II, Harper, *J.*, 1946, 892; Part IV, *loc. cit.*). Hydrolysis of this ester and condensation of the sodium salt with pyruvaldehyde in aqueous solution gave *cis*-3-hydroxy-*n*-dec-8-ene-2 : 5-dione, cyclised by aqueous sodium hydroxide to (±)-*cis*-*n*-pent-2-enylrethrolone (I*c*). This keto-alcohol was esterified with (+)-*trans*-chrysanthemoyl chloride (cf. Part V, *loc. cit.*) to give (±)-*cis*-*n*-pent-2-enylrethronyl (+)-*trans*-chrysanthemate, a true homologue of cinerin-I.

Dr. E. A. Parkin and Mr. A. A. Green of the Pest Infestation Laboratory have compared the insecticidal potencies of (±)-*cis*-*n*-pent-2-enylrethronyl (+)-*trans*-chrysanthemate and natural pyrethrins towards houseflies, over the concentration range 0·05—0·4% w/v in odourless distillate, by a modified Peet–Grady method (Parkin and Green, *Nature*, 1944, **154**, 16). Dr. Parkin reports that "there was no difference in toxicity between the two sets of corresponding solutions, either in knock-down in 10 minutes, or in kill in 24 hours." This homologue of cinerin-I, therefore, is comparable in toxicity towards houseflies to the (±)-allylrethronyl chrysanthemates, and the (±)-*n*-pent-2-enylrethronyl chrysanthemates are clearly worthy of further study.

Experimental.

Analyses are by micromethods. M. p.s are uncorrected. The infra-red absorption spectra were determined by one of us (L. C.) with a Grubb Parsons single-beam spectrometer coupled to a Brown recorder. Capillary films of pure liquid samples (*ca.* 5-μ. thickness) were used. We are indebted to Dr. W. C. Price for this facility.

trans-n-*Hept-5-en-2-one*.—*trans*-*n*-Pent-3-en-1-ol, b. p. 136—137°, n_D^{20} 1·4339, was prepared by the ring scission of *trans*-3-chloro-2-methyltetrahydrofuran in 73% yield and converted into its bromide (n_D^{20} 1·4696) as described previously (Crombie and Harper, *J.*, 1950, 1715).

To a mixture of acetic anhydride (10 g.) and anhydrous ether cooled to −70° in a Dewar flask the similarly cooled Grignard reagent from *trans*-*n*-pent-3-enyl bromide (7·45 g.) and mangesium (1·22 g.) in ether (25 ml.) was added dropwise during 30 minutes with good stirring. A white solid separated and after a further 2 hours' stirring the mixture was decomposed with aqueous ammonium chloride. After isolation, distillation gave *trans*-*n*-hept-5-en-2-one (3·38 g., 60%), b. p. 148—152°, n_D^{20} 1·4309. The 2 : 4-*dinitrophenylhydrazone*, prepared in ethanol–hydrochloric acid, crystallised from ethanol as yellow needles, m. p. 69—70° (Found: C, 52·7; H, 5·6. $C_{13}H_{16}O_4N_4$ requires C, 53·4; H, 5·5%).

The semicarbazone of a redistilled specimen of the ketone described in Part V was prepared in low yield in aqueous ethanol with semicarbazide hydrochloride and sodium acetate and on crystallisation from ethanol had m. p. 69—70°; no product was obtained by the use of semicarbazide hydrochloride and pyridine. The 2 : 4-dinitrophenylhydrazone, prepared as above and crystallised from ethanol and then from benzene–light petroleum (b. p. 60—80°), had m. p. 69—70°, not depressed on admixture with the above derivative (Found: C, 53·15; H, 6·1%).

n-*But*-2-*yn*-1-*ol*.—Technical grade 1 : 3-dichlorobut-2-ene (1 kg., 8 mols.; not redistilled) was added with stirring to boiling 2N-sodium carbonate (6 l.) and after refluxing for 6 hours the reaction mixture was steam-distilled. The product was separated from the distillate, dried (Na_2SO_4), and distilled to give 3-chlorobut-2-en-1-ol (62% mean of several runs), b. p. 100—102°/70 mm., n_D^{15} 1·4673. Hatch and Ballin (*J. Amer. Chem. Soc.*, 1949, **71**, 1039) recorded a 72% yield from purified 1 : 3-dichlorobut-2-ene, but Hatch and Chiola (*ibid.*, 1951, **73**, 360) have since obtained only a 63% yield.

3-Chlorobut-2-en-1-ol (533 g., 5 mols.), flake sodium hydroxide (230 g.), and water (450 ml.) were refluxed with stirring for 2 hours. The reaction mixture was steam-distilled, and the product separated from the distillate, combined with those from several runs, dried (K_2CO_3), and fractionally distilled through a 100 × 2·5-cm. helices-packed total-reflux variable take-off column. Fractions having b. p. 140·0—142·0° and n_D^{20} 1·4538—1·4534 were united and represented a 38% yield of *n*-but-2-yn-1-ol. Hatch and Nesbitt (*ibid.*, 1950, **72**, 727) recorded a 40% yield.

n-*But*-2-*ynyl Chloride*.—Phosphorus trichloride (85·5 g.) was added during 1 hour to a stirred mixture of *n*-but-2-yn-1-ol (105 g.) and technical-grade pyridine (39 ml.) cooled to −5°. After being stirred for a further 3 hours, the last hour at room temperature, the product was distilled from the reaction mixture and had b. p. 58—74°/200 mm. The crude products from several such runs were bulked, washed successively with water, sodium carbonate, hydrochloric acid, and water, dried (Na_2SO_4), and distilled through the column described above. Fractions having b. p. 101·0—103·0° and n_D^{20} 1·4592 were united and represented a 56% yield of *n*-but-2-ynyl chloride.

n-*Hept*-5-*yn*-2-*one*.—By the procedure of Part V (*J.*, 1950, 3552), on a 1-mole scale, *n*-but-2-ynyl chloride yielded n-*hept*-5-*yn*-2-*one* (37%), b. p. 58—63°/10 mm., n_D^{20} 1·4495 (Found: C, 75·6; H, 9·05. $C_7H_{10}O$ requires C, 76·35; H, 9·1%). The 2 : 4-*dinitrophenylhydrazone* crystallised as fine orange plates (from ethanol), m. p. 122·0—122·5° (Found: C, 54·0; H, 4·9; N, 19·05. $C_{13}H_{14}O_4N_4$ requires C, 53·8; H, 4·8; N, 19·35%).

The neutral oils from several runs, removed before acidification and warming to liberate the ketone, were bulked, dried, and fractionally distilled to give further *n*-hept-5-yn-2-one (equivalent to an additional 8% yield), b. p. 59—75°/10 mm., n_D^{20} 1·4500, identified by its 2 : 4-dinitrophenylhydrazone, m. p. 122° (and mixed m. p.), together with *ethyl* αα-*di*-n-*but*-2-*ynylacetoacetate* (2-*acetyl*-2-n-*but*-2′-*ynyl*-n-*hex*-4-*ynoate*), b. p. 100—101°/0·1 mm., n_D^{20} 1·4757 (Found: C, 71·1; H, 7·5. $C_{14}H_{18}O_3$ requires C, 71·4; H, 7·7%).

cis-n-*Hept*-5-*en*-2-*one*.—*n*-Hept-5-yn-2-one (68 g.) was shaken in hydrogen over pre-reduced 5% palladium–calcium carbonate (5·5 g.) in ethyl acetate until 14 l. had been absorbed. Fractional distillation then gave cis-n-*hept*-5-*en*-2-*one* (54 g., 78%), b. p. 43—48°/10 mm., 148—151°, n_D^{20} 1·432 (Found: C, 75·4; H, 11·0. $C_7H_{12}O$ requires C, 75·0; H, 10·8%). The *semicarbazone*, prepared as described above, crystallised from ethanol in superb plates, m. p. 124—125° (Found: C, 57·4; H, 8·95. $C_8H_{15}ON_3$ requires C, 57·5; H, 8·95%), while the 2 : 4-*dinitrophenylhydrazone*, also prepared as described

above, crystallised from ethanol as orange needles, m. p. 83·0—84·5° (Found: C, 53·3; H, 5·5. $C_{13}H_{14}O_4N_4$ requires C, 53·4; H, 5·5%).

(±)-cis-*Cinerolone*.—By the procedure of Part V *cis*-*n*-hept-5-en-2-one (48 g.) yielded *ethyl* cis-2-*keto*-n-*hept*-5-*ene*-1-*carboxylate* (56 g., 71%), b. p. 75—79°/0·1 mm., n_D^{20} 1·449 (Found: C, 65·15; H, 9·0. $C_{12}H_{18}O_3$ requires C, 65·2; H, 8·7%). This ester (46 g.) was shaken with 3% aqueous sodium hydroxide (330 ml.) during 72 hours and then the sodium salt was condensed with aqueous pyruvaldehyde (67 ml.) as described in Part IV (*J.*, 1950, 1152). Fractional distillation of the product gave *cis*-3-hydroxy-*n*-dec-8-ene-2 : 5-dione (16·8 g., 36%), b. p. 108—120°/0·3 mm., n_D^{20} 1·465—1·472, of which 14·8 g. were cyclised by shaking it with 3% aqueous sodium hydroxide (115 ml.) for 1 hour. Isolation of the product as described gave (±)-*cis*-cinerolone (5·5 g., 41%), b. p. 116—130°/0·2 mm., of which the middle runnings had n_D^{20} 1·513 (Found: C, 72·2; H, 8·75. Calc. for $C_{10}H_{14}O_2$: C, 72·25; H, 8·5%). Uptake on micro-hydrogenation in glacial acetic acid over Adams's catalyst: 2·96 mols. ($2\pi + 1C{:}O$); light absorption in ethanol: $\lambda_{max.}$ 2280 A., $\epsilon_{max.}$ 12,700. The semicarbazone had m. p. 199—201° (decomp.) when determined under the conditions previously described; a 1 : 1 mixture with naturally derived (±)-*cis*-cinerolone semicarbazone, m. p. 195—197° (decomp.), had m. p. 196—198° (decomp.).

Methyl cis-2-*Keto*-n-*oct*-5-*ene*-1-*carboxylate*.—*cis*-*n*-Hex-3-en-1-ol (186 g.), isolated as described by Crombie and Harper (*J.*, 1950, 873) from the same batch of tailings, was converted by Crombie and Harper's procedure (*J.*, 1950, 1715), with phosphorus tribromide (204 g.) at −25° to −30°, into *cis*-*n*-hex-3-enyl bromide (180 g., 60%), b. p. 138—140°, n_D^{20} 1·4729.

This bromide was stirred with potassium cyanide (120 g.) in ethylene glycol (500 ml.) for 2 hours at 100°, the reaction mixture poured into water, and the cyanide taken up in ether, dried (Na_2SO_4), and distilled to give *cis*-*n*-hex-3-enyl cyanide (112 g., 85%), b. p. 84—86°/25 mm., n_D^{20} 1·4352. The cyanide was refluxed in 20% aqueous potassium hydroxide (500 ml.) during 18 hours, the reaction mixture cooled, extracted with ether, and the aqueous layer acidified. The acid that separated was taken up in ether, dried (Na_2SO_4), and distilled to give *cis*-*n*-hept-4-enoic acid (108 g., 83%), b. p. 130—136°/30 mm., n_D^{20} 1·4416.

By the procedure of Part II (*J.*, 1946, 892) *cis*-*n*-hept-4-enoyl chloride (121 g.), b. p. 72—76°/35 mm., n_D^{20} 1·4500, prepared from the above acid in 89% yield by the use of thionyl chloride, was condensed with ethyl sodioacetoacetate (from 123 g. of acetoacetic ester) in ether, and then without distillation treated with cold methanolic sodium methoxide (550 ml., from 27 g. of sodium) to give methyl *cis*-2-keto-*n*-oct-5-ene-1-carboxylate (43 g., 33% for the two stages), b. p. 131—137°/30 mm., n_D^{20} 1·4524 (Found: C, 65·5; H, 9·3. Calc. for $C_{10}H_{16}O_3$: C, 65·2; H, 8·8%).

4-*Hydroxy*-3-*methyl*-2-cis-n-*pent*-2′-*enyl*cyclo*pent*-2-*en*-1-*one* [(±)-cis-n-*Pent*-2-*enylrethrolone*].—By procedure *A* of Part V methyl *cis*-2-keto-*n*-oct-5-ene-1-carboxylate (30 g.) was shaken with 10% aqueous sodium hydroxide (150 ml.) during 48 hours and the resulting solution of sodium salt condensed with aqueous pyruvaldehyde (35 ml.) at 35° during 6 hours. Isolation as described then gave cis-3-*hydroxy*-n-*undec*-8-*ene*-2 : 5-*dione* (9·0 g., 26%), b. p. 95—104°/0·1 mm., n_D^{20} 1·461—1·463. This was stirred with 10% aqueous sodium hydroxide (50 ml.) during 1 hour and the product isolated by the procedure of Part V. Distillation at 0·1 mm. gave several fractions, of which those having b. p. 100—114° and n_D^{20} 1·503—1·511 were bulked (2·16 g.) and redistilled to give (±)-*cis*-*n*-pent-2-enylrethrolone (1·60 g., 20%), b. p. 89—95°/0·03 mm., n_D^{20} 1·506—1·508.

(±)-cis-n-*Pent*-2-*enylrethronyl* (+)-trans-*Chrysanthemate*.—Following the procedure of Part V the reaction product from (±)-*cis*-*n*-pent-2-enylrethrolone (1·60 g.), (+)-*trans*-chrysanthemoyl chloride (1·60 g.), and pyridine (1·3 g.) in benzene (27 ml.) was distilled at 3×10^{-3} mm. and, after elimination of a forerun (0·20 g.), the *ester* was collected (0·66 g., 23%), b. p. 99·5—100·5°, n_D^{20} 1·4992—1·5003, of which the middle runnings had n_D^{20} 1·5000 (Found: C, 74·5; H, 9·05. $C_{21}H_{30}O_3$ requires C, 76·3; H, 9·1%). Light absorption in ethanol: $\lambda_{max.}$ 2270 A.; $\epsilon_{max.}$ 17,700.

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540. *Deoxypentose Nucleic Acids. Part II.* Evidence for a Labile Polymeric Linkage in Deoxypentose Nucleic Acids.*

By W. G. Overend, M. Stacey, and M. Webb.

Evidence is presented to show that some of the bonds in highly polymeric deoxypentose nucleic acids may involve labile phosphate residues attached at $C_{(1)}$ of the deoxypentofuranose constituents.

It was stated by Stacey, Overend, and Chong-fu Li (*Nature*, 1949, **163**, 538) that some of the highly labile bonds whereby the maximum macromolecular state of native deoxypentose nucleic acid is maintained involve $C_{(1)}$ of the deoxypentose moiety. Further the view was ventured that these might be highly labile glycosidic phosphate linkages. They based their ideas on the fact that highly polymerised deoxypentose nucleic acid isolated from soft herring roes by the well-established and elegant method of Mirsky and Pollister (*J. Gen. Physiol.*, 1946, **30**, 117) could be irreversibly depolymerised to some extent by dialysis for several days, and afforded a product which gave immediately an intense colour with Schiff's reagent (cf. Feulgen, *Z. physiol. Chem.*, 1924, **135**, 203). The dye so formed was completely non-soluble and non-diffusible; the reagent above the dyed nucleic acid remained colourless for more than 30 hours. It was also observed that the N/P ratio of the product was increased, but the purine content was retained. Continuation of studies recently reported (Overend and Webb, *J.*, 1950, 2746) has afforded results which can be interpreted on the basis of the ideas proposed by Stacey *et al.* (*loc. cit.*).

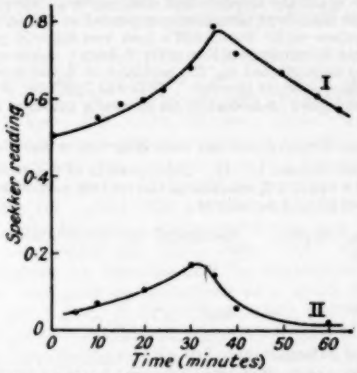
Herring-roe deoxypentose nucleic acid has been dialysed against water and the liquid outside the dialysis sac was collected and evaporated to reduce its volume. In the concentrate it was possible to demonstrate the presence of inorganic phosphate in very small amount. It corresponded to less than 2% of the total amount of phosphorus in the nucleic acid sample used. So far, despite careful search by the chromatographic technique (cf. Vischer and Chargaff, *J. Biol. Chem.*, 1948, **176**, 703, 715), we have been unable to show the presence of basic materials in the concentrate.

Recently (*Research*, 1949, **2**, 99; *J.*, 1950, 2746) we reported a study of the action of ox-pancreas deoxyribonuclease on deoxyribonucleic acid and showed that an initial depolymerisation of the substrate occurs, as evidenced by a rapid fall in viscosity, before cleavage of the nucleic acid into acid-soluble polynucleotides commences. The course of the enzyme reaction has now been followed, by using the supersensitive aldehyde reagent developed by Tobie (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 405) as a modification of Schiff's reagent (*Annal. Chem. Pharm.*, 1866, **140**, 102) (for use of this reagent, see Overend, *J.*, 1950, 2769). The result is shown in the figure. It is clear that, during the initial period when the rapid fall in viscosity occurs, there is an increase in the intensity of colour developed when Schiff's reagent is added. After this period has elapsed the intensity of the colour diminishes again. This point corresponds with the onset of formation of acid-soluble polynucleotides. Control experiments indicated lack of nucleosidase activity in the enzyme preparation used. Reference to the formulæ ($A \longrightarrow E$) indicates a possible explanation of the changes.

It is suggested that in the nucleic acid polymeric chain there occurs infrequently a 2-deoxy-D-ribofuranose unit (*A*) (for evidence that the sugar exists in nucleic acid in the furanose form see Lythgoe and Brown, *J.*, 1950, 1990) which does not carry a glycosidically bound purine or pyrimidine base. Instead the glycosidic linkage (*a*) is a labile phosphate bridge between two nucleic acid chains. In this way it would help to build up the macromolecular structure of the nucleic acid. Recent work with 2-deoxy-pentoses and -hexoses has emphasised the instability of the glycosidic linkage in sugars of this class, especially when they are in the furanose form (see Overend and Stacey, *J. Sci. Food Agr.*, 1950, **1**, 168, for a review of this evidence), and the work of Friedkin and Kalckar (*J. Biol. Chem.*, 1950, **184**, 437, 449) on 2-deoxy-D-ribose-1 phosphate has demonstrated the extreme lability of deoxypentose glycosidic phosphate linkages. When the enzymic depolymerisation commences this bond is hydrolysed and gives (*B*) which can exist in equilibrium with the aldehydo-form (*C*). The latter structure reacts with Schiff's reagent to give a coloured complex (Wieland and Scheuing, *Ber.*, 1921, **54**, 2527; see also Rumpf, *Ann. Chim.*, 1935, **3**, 327). As the depolymerisation proceeds the intensity of colour increases and when the depolymerisation of the nucleic acid is complete, the formation of acid-soluble polynucleotides commences by cleavage of the internucleotides linkages so that a structure

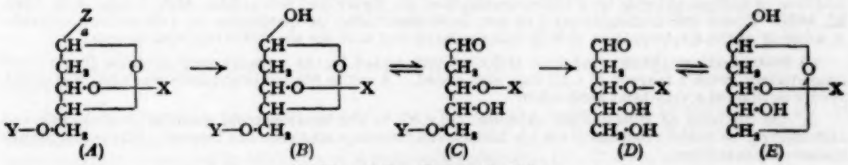
* Part I, *J.*, 1950, 2746.

represented by (*D*) is formed. In a molecule of this type the sugar moiety would co-exist in the aldehyde and pyranose forms, the latter being represented by (*E*). One of us (Overend, *loc. cit.*) has shown that when Schiff's reagent is added to *aldehydo*-2-deoxy-D-ribose co-existing in equilibrium with 2-deoxy-D-ribofuranose [(*B*) and (*C*) are the analogous examples in the nucleic acid molecule] the intensity of colour produced is much greater than when the reagent is added to an equilibrium mixture of the aldehyde and pyranose forms [(*D*) and (*E*) are the analogues in the example being discussed]. Consequently if during the enzymic degradation the Schiff's reagent is added when the reaction has reached the stage represented by (*D*) and (*E*) the intensity of colour developed will be less than if it is added at a stage represented by (*B*) and (*C*).



I. Thymus deoxypentose nucleic acid. *II. Herring-roe deoxypentose nucleic acid.*

Consequently a curve as shown in the figure would be expected. Recently Peacocke and Lee (personal communication; cf. Overend and Peacocke, *Trans. Faraday Soc.*, 1950, **46**, 790) by electrometric titrations have obtained quantitative data which can be interpreted in terms of an irreversible reaction with acid and alkali under very mild conditions (*i.e.*, pH 2 and 12 at 25°). Under the same conditions an increase in intensity of the colour developed with Schiff's reagent was observed. The latter data favour the existence of a labile linkage in the nucleic acid.



X, Y, and Z = Polynucleotides.

Finally we draw attention to an observation we have frequently noted. Several times we have encountered samples of deoxypentose nucleic acids, from both calf thymus gland and soft herring roes, which during deproteinisation cling tenaciously to traces of protein residue which can be detected by the Sakaguchi reaction. In such cases, when fairly vigorous methods are needed to remove these last protein traces, *e.g.*, brief autolysis at pH 3, prolonged dialysis, repeated shaking with chloroform and butanol, etc., the purified nucleic acid frequently reacts rapidly with Schiff's reagent and may have become depolymerised. Consequently the possibility must be considered that these labile glycosidic phosphate linkages may unite the nucleic acid to the protein component of the nucleoprotein at some sites, by linkages other than electrovalent linkages. However, this problem is being studied further. Some of our results appear to indicate that aldehyde groups are liberated when deoxyribonucleohistone is dissociated into deoxyribonucleic acid and histone, by dissolution in 1·0M- or 2·0M-sodium chloride.

Experimental.

The deoxyribonuclease was prepared according to the method described by Overend and Webb (*loc. cit.*), and the nucleic acids used were samples prepared by Mr. Laland, of this department, according to Mirsky and Pollister's method (*loc. cit.*).

A mixture of 1% (w/v) sodium thymonucleate (48 c.c.), veronal buffer (pH 6·92; 24 c.c.), 0·1M-magnesium sulphate (24 c.c.), and distilled water (12 c.c.) was heated at 37°, and then a 0·002% (w/v) solution of deoxyribonuclease (12 c.c.) preheated to 37° was added. At intervals of 5 minutes, aliquots (1 c.c.) of the digest were taken and diluted with distilled water (5 c.c.), and standard neutral supersensitive Schiff's reagent (Tobie, *loc. cit.*) (1 c.c.) was added. The colour was allowed to develop for exactly 15 minutes at 15° and then its intensity was measured in the Spekker photoelectric absorptiometer, Ilford filter No. 605 being used. The experiment was carried out with nucleic acid isolated from both calf thymus gland and soft herring roes. Reults are shown in the figure.

Qualitative Schiff's Test on Fibrous Sodium Deoxyribonucleate isolated from Herring Roe.—(1) *Dialysis.* In a typical experiment, a 1% solution of the sodium nucleate was prepared and shown to give no reaction with the Schiff's reagent while it had a slight positive Sakaguchi test. The solution was treated with a few drops of toluene to manitain sterility and dialysed in a Cellophane bag against running tap water. After 12 hours, a slight flocculent precipitate appeared in the solution, and the Sakaguchi test on the solution was now negative while the Schiff's test was slightly positive. On continuation of dialysis the Schiff's test became intensely positive after 4 days. After evaporation of the solution to small volume, the product was precipitated by the addition of 4 volumes of alcohol at pH 3 and was isolated in the form of a hygroscopic white powder. This was insoluble in water, gave positive tests for purine and pyrimidine bases, and gave immediately an intensely coloured insoluble complex on addition of Schiff's reagent.

In other experiments, traces of phosphate ion were detected in the dialysate.

(2) *Treatment with chloroform–butanol* (4 : 1). The removal of the residual protein was achieved by successively shaking 200 c.c. of a viscid 1% solution of the sodium salt of the nucleic acid with portions of chloroform (20 c.c.) and butanol (5 c.c.) as follows :

Treatment.	Chloroform complex.	Sakaguchi test (on solution).	Schiff's test (on solution).
1	+	+	−
2	+	+	−
3	+	−	−
4	trace	−	−
5	−	−	+ (develops slowly)

The product isolated was fibrous in form.

(3) *Brief acid treatment.* A sample of the fibrous sodium salt of the nucleic acid was precipitated from aqueous solution by the addition of ethanol (3 volumes) containing 1% of hydrochloric acid. The product was washed several times with alcohol. A sample was dissolved in a citrate buffer at pH 3 and heated at 100° for 3 minutes. After isolation the non-fibrous product gave an intensely positive Schiff test on being kept in the reagent for 30 minutes.

From numerous other qualitative observations it appeared that whenever obvious depolymerisation of deoxypentose nucleic acids had occurred the Schiff's test was easy to demonstrate.

The Changes accompanying Dissociation of Deoxyribonucleohistone.—Deoxyribonucleohistone was isolated from calf thymus gland by extraction with 0·002M-sodium arsenate and was precipitated by addition of sodium chloride to 0·14M-concentration (cf. Signer and Schwander, *Helv. Chim. Acta*, 1949, **32**, 853). Hence the nucleoprotein had not been dissociated by dissolution in 1·0M-sodium chloride. A solution of the nucleoprotein (0·8%) was prepared and used for the following experiments :

(i) 0·14M-Sodium chloride solution (4·0 c.c.) was added to the nucleoprotein solution (1 c.c.) and immediately Schiff's reagent (1 c.c.) was also added. A white fibrous precipitate was obtained which slowly developed a very faint pink colour.

(ii) On addition of 2·0M-sodium chloride (4·0 c.c.) to the nucleoprotein solution (1 c.c.), followed immediately by Schiff's reagent (1 c.c.), a bluish-pink fibrous precipitate was formed. The colour slowly became more intense.

(iii) On addition of Schiff's reagent (1 c.c.) to the nucleoprotein solution (1 c.c.) a *white* precipitate of the nucleoprotein was formed. This gradually developed an extremely faint pink colour.

(iv) The magnesium salt of the nucleoprotein was treated with a mixture of 2M-sodium chloride (2 c.c.) and Schiff's reagent (1 c.c.). The solid was instantaneously coloured bright pink.

(v) The magnesium salt of the nucleoprotein was dissolved in 2M-sodium chloride (3·0 c.c.), and Schiff's reagent (1 c.c.) was immediately added. A precipitate formed which rapidly became pink.

(vi) As a control, 2M-sodium chloride (3 c.c.) was added to Schiff's reagent (1 c.c.). No colour developed.

In experiments (iv) and (v) the insoluble magnesium salt of the nucleoprotein was prepared by adding 0·2% magnesium chloride solution (1 c.c.) to the nucleoprotein solution (1 c.c.). The precipitate was collected at the centrifuge (cf. Gilbert, Overend, and Webb, *Exp. Cell. Res.*, in the press). All the experiments were carried out under standard conditions in well-corked tubes.

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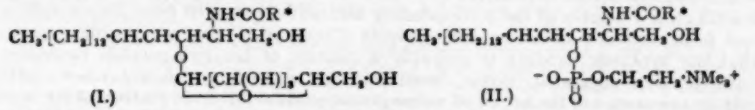
541. *The Sphingolipid Field. Part I. Synthesis of Racemic 2-Amino-octadecane-1 : 3-diols.*

By G. I. Gregory and T. Malkin.

The synthesis of a mixture of racemates of 2-amino-octadecane-1 : 3-diol is recorded, of which one form is known as dihydrosphingosine, obtained by hydrolysis of phospholipid sphingomyelin (Carter, Glick, Norris, and Phillips, *J. Biol. Chem.*, 1947, **170**, 285). The triacetyl, tribenzoyl, and *N*-acetyl derivatives of the synthetic amino-diol are described.

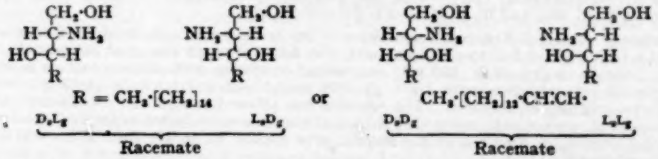
Whilst investigating the products formed by the hydrolysis of the phospholipid sphingomyelin, Thudichum (*J. pr. Chem.*, 1882, **25**, 19) isolated a base to which he gave the name sphingosine and the empirical formula $C_{17}H_{35}O_2N$. Later workers (Levene and Jacobs, *J. Biol. Chem.*, 1912, **11**, 547; Levene and West, *ibid.*, 1914, **16**, 549; Lapworth, *J.*, 1913, 1029) supported the C_{17} formula and also showed that sphingosine was a straight-chain compound containing two hydroxyl groups and a primary amino-group attached severally to the first three carbon atoms, and a double bond between the fourth and the fifth carbon atom; but Klenk and Diebold (*Z. physiol. Chem.*, 1929, **185**, 169; 1931, **198**, 25) adduced evidence, now accepted, in favour of a C_{18} chain, and proposed the formula 3-amino-octadec-4-ene-1 : 2-diol.

Carter, Glick, Norris, and Phillips (*J. Biol. Chem.*, 1947, **170**, 285) sought to confirm Klenk and Diebold's conclusions by investigating the degradation of dihydrosphingosine by periodic acid. Sphingosine, obtained by hydrolysis of a sphingomyelin–cerebroside mixture (Carter, Haines, Ledyard, and Norris, *J. Biol. Chem.*, 1947, **169**, 77; *ibid.*, **170**, 269), was *N*-benzoylated and reduced to give *N*-benzoyldihydrosphingosine, but this could not be induced to react with periodic acid, thus indicating the absence of the 1 : 2-glycol system postulated by Klenk and Diebold. On the other hand, evidence for a 2-amino-octadecane-1 : 3-diol structure was given by the formation of a benzylidene derivative of *N*-benzoyldihydrosphingosine and was supported by the reaction of periodic acid with dihydrosphingosine to yield hexadecanal, formaldehyde, formic acid, and ammonia. This work has also established the structure of sphingosine as 2-amino-octadec-4-ene-1 : 3-diol.



* The amino-group is acylated by a variety of acids, most frequently by lignoceric or α-hydroxylignoceric acid.

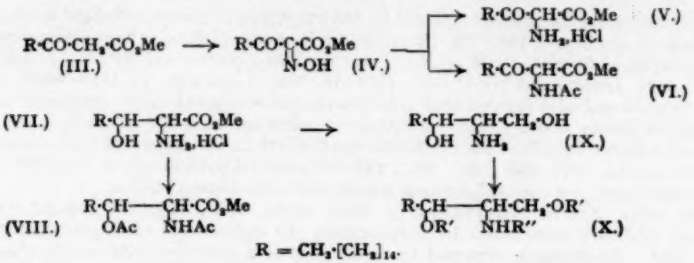
Dihydrosphingosine has been isolated directly from the hydrolysate of the cerebroside (I) fraction obtained from *Cysticerus fasciolaris* (Lesuk and Anderson, *ibid.*, 1941, **139**, 457) and, more recently, from the hydrolysate of a pure sphingomyelin (II) (Thannhauser and Boncoddo, *ibid.*, 1948, **172**, 141), where it occurs with the unsaturated base sphingosine.



The synthesis of dihydrosphingosine has not yet been described, although Carter (*ibid.*, 1947, **170**, 295) suggested that a possible synthetic route might lie through the intermediate 2-amino-3-hydroxyoctadecanoate esters,† and discussed the problems arising from the existence of two racemates of 2-amino-octadecane-1 : 3-diol. The configuration of the naturally occurring 2-amino-octadecane-1 : 3-diol, which we may continue to call dihydrosphingosine, has not yet been determined, and the present synthesis was undertaken in the hope of obtaining information on this.

† Geneva numbering (CO_2H = 1.)

Several routes to methyl 2-amino-3-keto-octadecanoate or its more stable hydrochloride having been investigated with no success, it was found possible to oximinate methyl 3-keto-octadecanoate (III) (Ställberg-Stenhagen, *Arkiv Kemi, Min., Geol.*, 1945, **20**, *A*, No. 19) in 55% yield. An attempt to reduce the oxime with lithium aluminium hydride gave a mixture which appeared to be long-chain amines; however, attempts to separate a pure substance were unsuccessful (cf. the attempted reduction of methyl α-oximinofuroylacetate by Hayes and Gever, *J. Org. Chem.*, 1951, **16**, 269). Stepwise reduction was next employed; first, the oxime (IV) was reduced with palladium–charcoal in the presence of 2 mols. of hydrogen chloride, to prevent the formation of secondary amines (cf. Hartung and Hamlin, *J. Biol. Chem.*, 1942, **145**, 349). The product was shown to be methyl 2-amino-3-keto-octadecanoate hydrochloride (V) which gave an acetyl derivative (VI), identical with the product obtained



by reductive acetylation of the oxime. Reduction of the hydrochloride (V) over platinum oxide gave methyl 2-amino-3-hydroxyoctadecanoate hydrochloride (VII) in good yield. The *NO*-diacetyl derivative (VIII) was prepared and was identical with the product obtained by a similar reduction followed by acetylation, of methyl 2-acetamido-3-keto-octadecanoate (VI). The final stage was carried out by reducing the free base, methyl 2-amino-3-hydroxy-octadecanoate, with lithium aluminium hydride to give 2-amino-octadecane-1 : 3-diol (IX), isolated as the hydrochloride. The *N*-acetyl (X; R′ = H, R″ = Ac), triacetyl (X; R′ = R″ = Ac), and tribenzoyl derivatives (X; R′ = R″ = Bz) were prepared and had melting points within a few degrees of the corresponding derivatives prepared from dihydrosphingosine obtained from naturally occurring sphingolipids (Carter *et al.*, *loc. cit.*, p. 269), despite the fact that the synthetic product is probably a mixture of the two possible racemates. It has, however, been suggested (Fodor, Bruckner, Kiss, and Öhegyi, *J. Org. Chem.*, 1949, **14**, 337) that the orientation of the keto- and oxime groups predetermines the position of the hydroxy- and amino-groups in the reduction product, leading to the formation of one racemate only.

The salts (V) and (VII), and the hydrochloride of (IX) were submitted to paper-partition chromatography and found to have R_F values 0·12, 0·88, and 0·73 respectively. Under similar conditions, a sample of natural dihydrosphingosine hydrochloride had R_F 0·72.

Experimental.

Methyl 3-Keto-octadecanoate (III).—This was prepared according to Ställberg-Stenhagen's method (*loc. cit.*) in 55% yield, and had m. p. 48·5—49° (from methanol).

Oximination of Methyl 3-Keto-octadecanoate.—Of the several methods tried for introduction of an oximino-group into methyl 3-keto-octadecanoate, the following was the most successful. A solution of hydrogen chloride in dry ether (150 ml.) was added dropwise, with stirring, to an ice-cooled solution of methyl 3-keto-octadecanoate (12·5 g., 0·04 mole) and butyl nitrite (8·25 g., 0·08 mole) in dry ether (60 ml.) during 45 minutes. The mixture was allowed to warm to room temperature during 1 hour, then poured into ice-cold water (300 ml.), and the ethereal solution separated, washed free from acid, and dried ($MgSO_4$). Removal of the solvent gave a solid, m. p. 57—59°. This was not purified, but was reduced directly.

When the oximination was carried out by passing a rapid stream of gaseous hydrogen chloride into an ethereal solution of methyl 3-keto-octadecanoate and butyl nitrite, a solid, m. p. 88—90°, separated and was removed by filtration. Recrystallisation from alcohol, ether, and finally light petroleum (b. p. 60—80°) gave *3-keto-2-oximino-octadecanoic acid*, m. p. 91—91·5° (Found : C, 66·2; H, 9·7; N, 4·3. $C_{18}H_{33}O_4N$ requires C, 66·1; H, 10·1; N, 4·3%).

Methyl 2-Amino-3-keto-octadecanoate Hydrochloride (V).—The crude oximination product (m. p. 57—59°) in ethyl alcohol (50 ml.) was shaken with hydrogen at 100 lb./sq. in., palladium–charcoal (2 g.; 10%; Ott and Schröter, *Ber.*, 1927, **60**, 633), palladium chloride (0·4 g.), and concentrated hydrochloric acid (6 ml.; 35%) for 6 hours. The precipitated crystalline solid was redissolved by

addition of a further 150 ml. of alcohol and warming; the catalyst was removed by filtration and the filtrate evaporated to dryness. The solid was refluxed with dry ether for 30 minutes, filtered off, and recrystallised twice from ethyl alcohol, to give *methyl 2-amino-3-keto-octadecanoate hydrochloride*, m. p. 115° (Found: C, 62·2; H, 10·2; N, 4·1; Cl, 9·8. $C_{19}H_{38}O_3NCl$ requires C, 62·7; H, 10·5; N, 3·9; Cl, 9·7%). The yield, from methyl 3-keto-octadecanoate, was 7·4 g. (55%). This compound gives a red-violet colour with ninhydrin and a cherry-red colour with alloxan.

The hydrochloride (1 g.), sodium acetate (0·1 g.; anhydrous), and acetic anhydride (5 ml.), heated at 100° for 1 hour, cooled, and poured into ice-cold water (100 ml.), gave an oil which quickly solidified. Recrystallised from alcohol and then acetone the *N-acetyl* derivative gave rosettes, m. p. 85° (Found: C, 68·1; H, 10·4; N, 3·4. $C_{21}H_{39}O_4N$ requires C, 68·2; H, 10·6; N, 3·8%). When a melted specimen was viewed under a polarising microscope, large feathery spherulites were seen. The 2 : 4-*dinitrophenylhydrazone* of this derivative separated from alcohol and was recrystallised from ethyl acetate; it had m. p. 114° (Found: C, 58·75; H, 7·6; N, 12·5. $C_{27}H_{43}O_7N_5$ requires C, 58·95; H, 7·9; N, 12·75%). It is interesting that this analysis corresponds to the open-chain derivative, whereas ethyl α-acetamidoacetoacetate forms a pyrazolone derivative with 2 : 4-dinitrophenylhydrazine (Albertson *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 1150).

Methyl 2-acetamido-3-keto-octadecanoate was also prepared directly from the crude oximination product by reductive acetylation as follows. To the crude oximination product (2·0 g.) (m. p. 57—59°) in acetic acid (20 ml.)–acetic anhydride (12 ml.), zinc dust (3 g.) was added with stirring during 20 minutes, the temperature rising to about 40°. The mixture was stirred at 50° for 3 hours and then at room temperature for 2 hours, after which water (30 ml.) was added and the zinc removed by filtration. The zinc cake was washed with chloroform (3 × 20 ml.) and this extract added to the filtrate, the chloroform layer separated, washed with water followed by saturated sodium hydrogen carbonate, and dried ($MgCl_2$), and the solvent removed. The solid was recrystallised from acetone eight times, whereafter it had m. p. 83—84°; the mixed m. p. with the previously prepared specimen was 84°.

Methyl 2-Amino-3-hydroxyoctadecanoate Hydrochloride (VII).—Methyl 2-amino-3-keto-octadecanoate hydrochloride (5·5 g.) in ethyl alcohol (200 ml.) was shaken with hydrogen at 100 lb./sq. in. at room temperature for 10 hours with platinum oxide (Adams's catalyst; 0·25 g.). The catalyst was removed by filtration, a further quantity of fresh platinum oxide added, and the reduction continued for a further 10 hours. The solution, after removal of catalyst, was concentrated to 50 ml. and cooled to 0°, whereupon some unchanged material crystallised and was recovered. On evaporation of the solution to dryness, 4·7 g. of material were obtained and refluxed with dry ether for 30 minutes; removal of the solid (4·5 g.) by filtration gave *methyl 2-amino-3-hydroxyoctadecanoate hydrochloride*, m. p. 122—125° (82%) (Found: C, 62·3; H, 10·9; N, 4·0; Cl, 10·0. $C_{19}H_{40}O_3NCl$ requires C, 62·3; H, 11·0; N, 3·8; Cl, 9·7%). This material gave a red-violet colour with alcoholic ninhydrin.

This hydrochloride (0·5 g.), with sodium acetate and acetic anhydride, gave *methyl 2-acetamido-3-acetoxyoctadecanoate*, m. p. 103·5—104·5° after recrystallisation from ethyl alcohol and acetone (Found: C, 66·4; H, 10·5; N, 3·7. $C_{23}H_{43}O_5N$ requires C, 66·8; H, 10·5; N, 3·4%). When viewed under the polarising microscope, a melted layer gave very large spherulites easily distinguishable from methyl 2-acetamido-3-keto-octadecanoate.

Reduction of Methyl 2-Acetamido-3-keto-octadecanoate.—A solution of the ester (0·5 g.) in ethyl alcohol (30 ml.) was shaken with platinum oxide (0·05 g.) and hydrogen at 100 lb./sq. in. for 10 hours; the catalyst was removed, a further quantity added (0·05 g.), and the hydrogenation continued for a further 10 hours. Removal of the catalyst and concentration of the solution gave a crystalline solid, m. p. 80—83°. This was acetylated in the usual manner with sodium acetate and acetic anhydride to give a product, m. p. 92—95°. Two recrystallisations from alcohol and one from acetone gave methyl 2-acetamido-3-acetoxyoctadecanoate, m. p. 102·5—103·5°; the mixed m. p. with a sample prepared as above was 103°.

2-Amino-octadecane-1 : 3-diol (IX).—Methyl 2-amino-3-hydroxyoctadecanoate hydrochloride (3·7 g., 0·01 mole) was shaken with ether (30 ml.) and a 5% aqueous solution of sodium acetate (100 ml.) containing one equivalent of sodium hydroxide (10 ml. of N.) until all the solid had disappeared. The ethereal solution was then separated and washed repeatedly with dilute aqueous alcohol until free from alkali. After being dried, the ether was removed, leaving the free base, methyl 2-amino-3-hydroxyoctadecanoate (3·0 g., 90%). The base was added in sodium-dried ether (20 ml.) dropwise with stirring to a solution of lithium aluminium hydride (1·15 g., 0·03 mole) in anhydrous ether (30 ml.) during 15 minutes. The reaction mixture was refluxed with stirring for 4 hours, after which water (10 ml.) was carefully added to decompose excess of lithium aluminium hydride. The ethereal layer was separated and shaken with 10% sodium hydroxide solution (20 ml.). A solid appeared at the interface and was removed by filtration; this appears to be sodium 2-amino-3-hydroxyoctadecanoate, obtained by hydrolysis of the original methyl ester. The ethereal solution was washed with water and dried ($MgSO_4$). Dry hydrogen chloride was passed in until precipitation was complete. Recrystallisation of the product gave 0·9 g. (30%) of *2-amino-octadecane-1 : 3-diol hydrochloride* (Found: C, 63·7; H, 11·5; N, 4·5; Cl, 10·8. $C_{18}H_{40}O_2NCl$ requires, C, 64·0; H, 11·9; N, 4·2; Cl, 10·5%). This product is probably a mixture of the two racemates.

A further quantity of amino-diol was recovered as the *N*-acetyl derivative (see below) from the residues of the reduction experiment, the total yield (from methyl 2-amino-3-hydroxyoctadecanoate) being 45%.

2-Acetamido-octadecane-1 : 3-diol (X; R′ = H, R″ = Ac).—To 2-amino-octadecane-1 : 3-diol hydrochloride (0·4 g.) in ether (30 ml.)–10% aqueous sodium hydroxide (20 ml.), acetic anhydride (0·3 g.) was added in three portions with cooling and shaking, a precipitate being formed in the ethereal layer. The precipitate was dissolved by addition of methanol, and the ether–methanol solution washed free

from alkali, dried, and concentrated to 5 ml., whereupon a precipitate formed; this was removed by filtration and recrystallised from methyl alcohol, to give 2-*acetamido-octadecane*-1 : 3-*diol*, m. p. 123—125° (Found: C, 70·0; H, 11·8; N, 4·1. $C_{20}H_{41}O_3N$ requires C, 69·9; H, 12·0; N, 4·1%). A melted specimen showed characteristic large spherulites when viewed under the polarising microscope. *N*-Acetyldihydrosphingosine, prepared by Carter *et al.* (*loc. cit.*, p. 269) from dihydrosphingosine sulphate obtained by hydrolysis of sphingolipids, had m. p. 126°.

Both 2-amino-octadecane-1 : 3-diol hydrochloride and 2-acetamido-octadecane-1 : 3-diol, when acetylated in the usual manner with acetic anhydride and sodium acetate, gave 2-*acetamido*-1 : 3-*diacetoxyoctadecane*, m. p. 98—100° (Found: C, 67·7; H, 10·7; N, 3·1. $C_{24}H_{45}O_5N$ requires C, 67·4; H, 10·6; N, 3·3%). Carter gives 102—103° as the m. p. of triacetyldihydrosphingosine.

The amino-diol (from 0·2 g. of the hydrochloride), benzoylated in pyridine (5 ml.) with benzoyl chloride (0·35 g.) at room temperature for 3 hours, gave an oil which quickly solidified. Three recrystallisations from ethyl alcohol gave the *tribenzoyl* derivative, m. p. 138—140° (Found: C, 76·3; H, 8·1; N, 2·4. $C_{39}H_{41}O_6N$ requires C, 76·4; H, 8·4; N, 2·3%). Carter records the m. p. of tribenzoyldihydrosphingosine as 144°.

Paper-partition Chromatography of the Hydrochlorides.—Alcoholic solutions (5%) of methyl 2-amino-3-keto- (V) and methyl 2-amino-3-hydroxy-octadecanoate hydrochloride (VII), and 2-amino-octadecane-1 : 3-diol hydrochloride were spotted, according to the technique of Consden, Martin, and Gordon (*Biochem. J.*, 1944, **38**, 224), on sheets of Whatman No. 1 filter paper. The solvent used was butanol–ethanol–water (40 : 19 : 11). Before the solvent had reached the bottom, the paper was removed, dried, and sprayed with a 0·2% alcoholic solution of ninhydrin. On warming, the methyl 2-amino-3-keto-octadecanoate hydrochloride was observed as purple spots (R_F = 0·12), and the 3-hydroxy-ester gave purple spots if left at room temperature for some time (R_F = 0·88). 2-Amino-octadecane-1 : 3-diol hydrochloride is best detected by spraying the chromatogram with ammoniacal silver nitrate, which gives brown spots (R_F 0·73). A specimen of dihydrosphingosine hydrochloride, obtained from the sphingolipids of beef brain, was subjected to paper-partition chromatography under similar conditions and had R_F 0·72.

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THE UNIVERSITY, BRISTOL. [*Received, May 29th*, 1951.]

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542. *Infra-red Absorption of the Carbonyl Group. Part I. Diacyl Peroxides, Per-esters, and Per-acids.*

By W. H. T. DAVISON.

Infra-red carbonyl absorption frequencies are reported for 18 symmetrical diacyl peroxides, six unsymmetrical diacyl peroxides, five per-esters, and perbenzoic acid. Characteristic carbonyl frequencies are given for diacyl peroxides and per-esters. Correlations are established between carbonyl frequencies of these compounds, and those of the corresponding acids, and also with Hammett's σ factors and thus with decomposition rates of benzoyl peroxides.

ALTHOUGH peroxides are of considerable interest in catalysed polymerisation and in the autoxidation and degradation of rubber, comparatively little infra-red or Raman work has been reported on them. In particular the O–O stretching frequency (ν_{O-O}) has not been assigned with certainty, although it has been variously and tentatively assigned by Shreve, Heether, Knight, and Swern (*Anal. Chem.*, 1951, **23**, 282) and Leadbeater (*Compt. rend.*, 1950, **230**, 829). There is, moreover, some reason to consider that there should be no very characteristic ν_{O-O}, caused by interaction with the adjacent ν_{O-O}. Unless the C–O–O angle is nearly 90°, considerable interaction of vibrations is to be expected (Herzberg, "Infra-red and Raman Spectra of Polyatomic Molecules," Van Nostrand, 1945, p. 199). A second difficulty is that, in hydrogen peroxide, the only peroxide for which a definite assignment has been made, the O–O stretching vibration has a weak infra-red absorption (Giguere, *J. Chem. Physics*, 1950, **18**, 88), which suggests that it may also be weak in other symmetrical peroxides; it is seldom possible to obtain simple peroxides of sufficient purity to justify the assignment of weak absorptions.

For a structural diagnosis of the benzoyl and acetyl derivatives of 1-hydroxy*cyclo*hexyl 1-hydroperoxide carried out for Dr. W. Cooper (*J.*, 1951, 1340) characteristic carbonyl frequencies ($\nu_{C=O}$) were established for diacyl peroxides and per-esters, and differentiated from those of other carbonyl compounds. In the course of this work, marked correlations were observed

between $\nu_{C=O}$ values of corresponding diacyl peroxides, per-esters, and acids. These, together with the work of Flett (*Trans. Faraday Soc.*, 1948, **44**, 767) on the relationship of $\nu_{C=O}$ and reaction rates of a series of benzoic acids, suggested that similar relationships might exist in the case of peroxides, and this would shed some light on relative strengths of peroxide bonds.

Experimental.

Apart from those which were commercially available, all the diacyl peroxides and per-esters were prepared by Dr. W. Cooper. The only evidence of purity of the diacetyl peroxide was the simplicity of its spectrum. Perbenzoic acid as prepared contained some benzoic acid which was mainly removed by two short-path vacuum distillations with a Washburn molecular still. Other reference compounds used were of normal laboratory standard. The carbonyl absorption is sufficiently strong that no interference was experienced from impurities, other than compounds either possessing, or hydrogen-bonded with, carbonyl groups.

Table I.

Symmetrical diacyl peroxides (aliphatic).

		$\nu_{C=O}$ (cm.$^{-1}$).				
	Solvent.	*A*.	*B*.	Mean.	Split.	F_I.
Acetyl	CCl_4	1820 ± 1	1796 ± 1	1808 ± 1	24 ± 2	6470
Butyryl	—	1816 ± 4	1784 ± 2	1800 ± 3	(32 ± 6)	6570
Octanoyl	—	1812 ± 1	1786 ± 1	1799 ± 2	26 ± 3	6610
Lauroyl	CCl_4	1811 ± 1	1786 ± 1	1798·5 ± 1	25 ± 2	—
Myristoyl	CCl_4	1811 ± 1	1785 ± 1	1798 ± 1	26 ± 2	—
Palmitoyl	CCl_4	1812 ± 1	1786 ± 1	1799 ± 1	26 ± 2	—
Stearoyl	CCl_4	1811 ± 1	1786 ± 1	1798·5 ± 1	25 ± 2	—

Average split (excl. butyryl), 25·3 cm.$^{-1}$.

The spectrophotometer used was a standard single-beam Grubb–Parsons instrument with sodium chloride prism; 0·1-mm. slits were used, except with Nujol mulls, for which larger slits were used. All spectra were plotted as percentage transmissions, and absorption frequencies were corrected for the atmospheric-water absorption at 1699·5 cm.$^{-1}$. Sharp absorptions could be read to about 1 cm.$^{-1}$, and estimated errors are given in the results (Tables I—IV). These estimates of accuracy are applicable within the series of results in this paper, but a small systematic error may arise from possible inaccuracies of the calibration curve. To minimise solvent shifts, carbon tetrachloride (" AnalaR ") was used where possible.

Table II.

Symmetrical diacyl peroxides (aromatic).

		$\nu_{C=O}$ (cm.$^{-1}$).					
	Solvent.	*A*.	*B*.	Mean.	Split.	F_I.*	σ.
Phthaloyl	Nujol	1805 ± 2	1783 ± 2	1794 ± 2	22 ± 4	4000	—
	Dioxan	1800 ± 5	1784 ± 2	1792 ± 4	26 ± 7	—	—
p-Nitrobenzoyl	$C_2H_4Cl_2$	1799 ± 2	1779 ± 1	1789 ± 2	20 ± 3	4640	0·778
	CCl_4	1804 ± 1	1782 ± 1	1793 ± 1	22 ± 2	—	—
m-Nitrobenzoyl	$C_2H_4Cl_2$	1800 ± 2	1777 ± 1	1788·5 ± 2	23 ± 3	4690	0·710
m-Chlorobenzoyl	,,	1797 ± 2	1773 ± 2	1785 ± 2	24 ± 4	5200	0·373
p-Iodobenzoyl	,,	1792 ± 2	1771 ± 1	1781·5 ± 2	21 ± 3	—	0·276
p-Bromobenzoyl	,,	1793 ± 1	1771 ± 1	1782 ± 1	22 ± 2	5420	0·232
p-Chlorobenzoyl	,,	1787 ± 2	1765 ± 1	1776 ± 2	22 ± 3	5500	0·227
p-Fluorobenzoyl	,,	1789 ± 1	1768 ± 1	1778·5 ± 1	21 ± 2	5280	0·062
Benzoyl	CCl_4	1792 ± 1	1771·5 ± 1	1782 ± 1	20 ± 2		
	$C_2H_4Cl_2$	1789 ± 2	1767 ± 1	1778 ± 2	22 ± 3	5690	0
	$CH_3{\cdot}NO_2$	1788 ± 2	1766 ± 1	1777 ± 2	22 ± 3		
p-Toluoyl	$C_2H_4Cl_2$	1785 ± 2	1763 ± 2	1774 ± 2	22 ± 4	5960	−0·170
p-Methoxybenzoyl	,,	1780 ± 2	1758 ± 2	1769 ± 2	22 ± 4	6120	−0·268

Average split, 22 cm.$^{-1}$.

* For definition of F_I see p. 2461.

Table III.

Mixed or unsymmetrical benzoyl peroxides.

	$\nu_{C=O}$ (cm.$^{-1}$).*			
	A.	*B*.	Mean.	Split.
Acetyl benzoyl	1811 ± 1	1779 ± 1	1795 ± 1	32 ± 2
Benzoyl stearoyl	1811 ± 2	1786 ± 1	1798·5 ± 2	25 ± 3
Benzoyl *p*-nitrobenzoyl	1797 ± 1	1773 ± 1	1785 ± 1	24 ± 2
Benzoyl *m*-nitrobenzoyl	1796 ± 2	1771·5 ± 1	1784 ± 2	24·5 ± 3
Dibenzoyl phthaloyl	1801 ± 1	1776 ± 1	1788·5 ± 2	25 ± 3
Adipyl dibenzoyl	1808 ± 2	1775 ± 1	1791·5 ± 2	33 ± 3

* All these were determined in CCl_4 solutions.

TABLE IV.

Per-esters and per-acid.

	Solvent.	$\nu_{C=O}$ (cm.$^{-1}$).	Other $\nu\nu$.
tert.-Butyl peracetate	—	1783 ± 2	—
tert.-Butyl perbenzoate	—	1758 ± 2	—
Di-*tert.*-butyl perphthalate	CCl_4	1772 ± 2	—
" *tert.*-Butyl perphthalic acid "	Nujol	1771 ± 1	1705, 1685, 1674
(*tert.*-Butyl *o*-carboxybenzoyl peroxide)	Dioxan	1776 ± 1	1730 ± 1
" *tert.*-Butyl permaleic acid "			
(*tert.*-Butyl β-carboxyacrylyl peroxide)	Dioxan	1776 ± 1	1737 ± 3
Perbenzoic acid	CCl_4	1732 ± 1	1696 ± 2

RESULTS AND DISCUSSION.

Symmetrical Diacyl Peroxides.—All of these showed a pair of strong carbonyl absorptions, the stronger being rather sharper and at a lower frequency (Fig. 1). The seven aliphatic diacyl peroxides examined had absorptions at 1811—1820 and 1784—1796 cm.$^{-1}$ with an average split of 25·3 cm.$^{-1}$ (Table I). Eleven aromatic diacyl peroxides had absorptions at 1780—1805 and 1758—1784 cm.$^{-1}$, with an average split of 22 cm.$^{-1}$ (Table II). The intensities of the two bands observed are not dependent upon concentration and are not, therefore, connected with any form of association. Coupling between the identical characteristic stretching vibrations of the two carbonyl bonds causes a frequency splitting, and gives rise to a pair of absorptions on either side of the " free " or unperturbed frequency (Herzberg, *op. cit.*). The mean of the two observed frequencies gives the characteristic frequency (when the split is small), and the split is a measure of the efficiency of the coupling. Thus diacyl anhydrides show a similar pair of absorptions (Thompson, *J.*, 1948, 328) though with a larger split (average 65 cm.$^{-1}$) (Modest and Szmuszkovicz, *J. Amer. Chem. Soc.*, 1950, **72**, 577; Davison, unpublished work) resulting from the shorter coupling link. The carbonyl absorptions of anhydrides may also be differentiated from those of diacyl peroxides by their contours, the stronger and sharper absorption of the pair being at a higher frequency (Fig. 1).

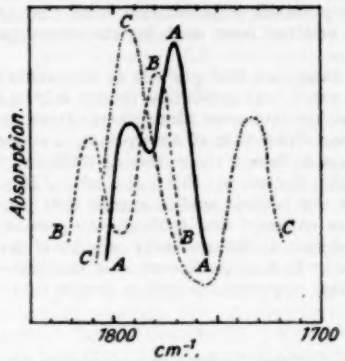
Correlations between $\nu_{C=O}$ of diacyl peroxides, per-esters, and per-acids indicate that this characterisation may need considerable extension towards higher frequencies. Linear extrapolation (Fig. 3) would lead to frequencies of 1867 and 1840 cm.$^{-1}$ for trichloroacetyl peroxide, but such extrapolation probably gives too high a frequency in these ranges.

Unsymmetrical Acyl Benzoyl Peroxides.—These similarly showed a pair of strong carbonyl absorptions of similar intensity and contour to those of the symmetrical compounds, but at 1796—1811 and 1771—1786 cm.$^{-1}$ (Table III). In unsymmetrical compounds R·O·OBz (where R is an acyl group) the two carbonyls will in general have different " free " or unperturbed frequencies. Thus acetyl benzoyl peroxide gives one absorption at 1811 cm.$^{-1}$, which can be assigned to the acetyl group (cf. 1808 cm.$^{-1}$, mean $\nu_{C=O}$ frequency of diacetyl peroxide), and one at 1779 cm.$^{-1}$ which is assigned to the benzoyl group (cf. 1782 cm.$^{-1}$, mean $\nu_{C=O}$ for dibenzoyl peroxide). In all these cases coupling causes some interaction and frequency splitting, which is greater when the " free " frequencies are closer. In this case a displacement of 3 cm.$^{-1}$ may be attributed to resonance splitting, and in the limiting case of dibenzoyl peroxide, coupling alone accounts for a split of 21 cm.$^{-1}$.

In Fig. 2, $\nu_{C=O}$ of peroxides R·O·OBz are plotted against their $\nu_{C=O}$ means and against the mean $\nu_{C=O}$ of peroxides R·O·OR. Where spectra were measured in ethylene dichloride, 4 cm.$^{-1}$ has been added to bring them into line with frequencies in carbon tetrachloride. Broken lines represent unperturbed frequencies (on the assumption that they are unaffected by replacement of Bz by R), and the chain line is the mean of these unperturbed frequencies. The full curves represent the form of theoretical interaction curves, fitted to the experimental points (Herzberg, *op. cit.*, Fig. 70, p. 216). With the exception of benzoyl stearoyl peroxide [shown thus ()], agreement is reasonable, and is consistent with resonance splitting and the assumption that the characteristic frequencies of the carbonyl groups are independent of the nature of the more remote acyl group. This assumption is consistent with the general decrease of influence with distance (cf. Watson, " Modern Theories of Organic Chemistry," O.U.P., 1941, p. 28) and direct spectroscopic evidence that carbonyl frequencies of esters are relatively independent of the nature of the alcoholic group (Hartwell, Richards, and Thompson, *J.*, 1948, 1436).

As discussed in the case of symmetrical peroxides, the characteristic frequencies of 1796—1811 and 1771—1786 cm.$^{-1}$ need some extension to allow for unsymmetrical peroxides such

FIG. 1.



A. Benzoyl peroxide. B. Acetyl benzoyl peroxide. C. Benzoic anhydride.

FIG. 3.

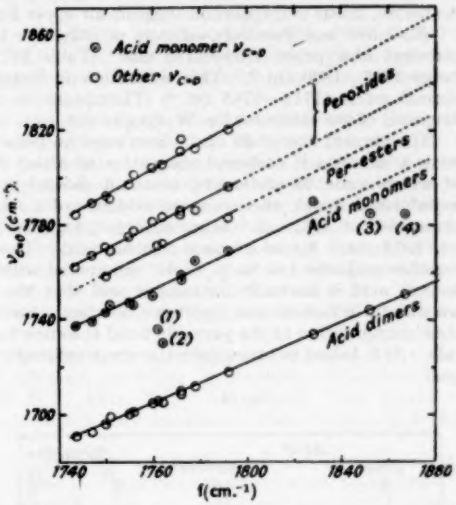
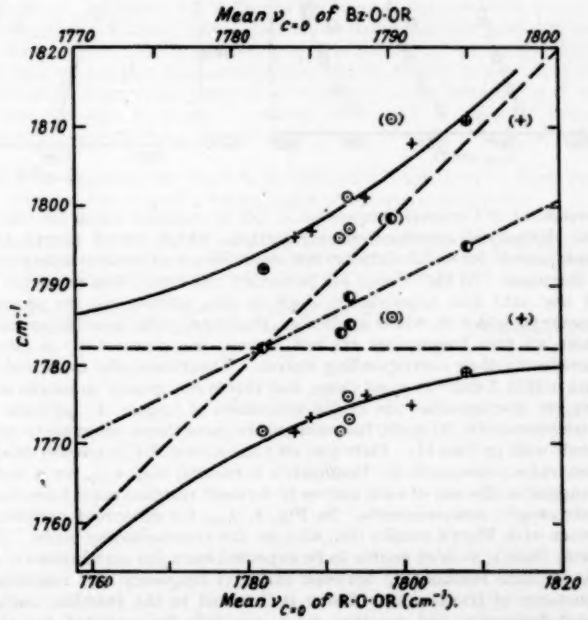


FIG. 2.



\+ *Observed against mean* Bz·O·OR.
○ ,, ,, *mean* R·O·OR.
◐ *Mean* Bz·O·OR *against mean* R·O·OR.

——— *Unperturbed (theoretical).*
– – – – *Theoretical mean* Bz·O·OR.
— · — · — *Theoretical.*

as benzoyl trichloroacetyl peroxide which would be expected to show an absorption at 1782 cm.$^{-1}$ (benzoyl) and one at considerably higher frequency assigned to the trichloroacetyl group. As before, linear extrapolation suggests an upper limit to this at about 1854 cm.$^{-1}$.

Per-esters and Per-acids.—Esters of only five per-acids were available and these show a carbonyl absorption 1758—1783 cm.$^{-1}$ (Table IV), which probably extends over the wider range 1747—1820 cm.$^{-1}$. This characteristic frequency is clearly differentiated from that of normal esters (1715—1765 cm.$^{-1}$) (Thompson, *loc. cit.*) and has been used for the structural diagnosis of per-esters for Dr. W. Cooper (*loc. cit.*).

The general rise of 36 cm.$^{-1}$ from ester to per-ester suggested that perbenzoic acid should have a monomeric carbonyl absorption at about 1780 cm.$^{-1}$. At a dilution (about 0·003M.) at which some 30 carboxylic acids all showed measurable monomer absorptions (Davison, unpublished work), there was no evidence of a monomer–dimer pair of absorptions, a single absorption at 1732 cm.$^{-1}$ being observed, together with a hydroxyl absorption at 3250 cm.$^{-1}$ (cf. 3551 cm.$^{-1}$ for monomeric benzoic acid; Thompson, quoted by Flett, *loc. cit.*). This, together with the low m. p. of 41° (compared with 122° for benzoic acid), suggests that perbenzoic acid is normally monomeric and that the lower carbonyl and hydroxyl frequencies are due to intramolecular hydrogen bonding. Such chelation is dimensionally possible if the skew configuration of the peroxide bond is somewhat nearer to a *cis*-configuration in this molecule. It is hoped to investigate the spectrum and structure of perbenzoic acid in greater detail later.

FIG. 4.

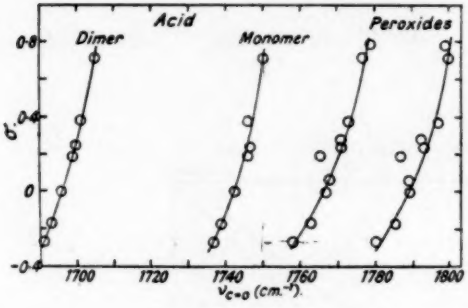
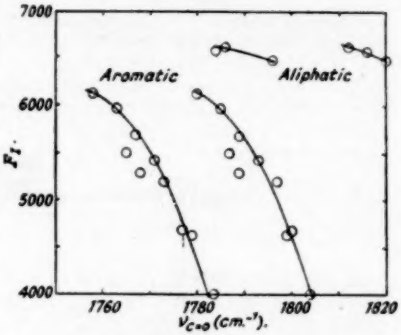


FIG. 5.



General Correlations of Carbonyl Frequencies.—Let us consider which correlations are likely to be of value. Empirical spectroscopic correlations which would permit the forecast of frequencies of compounds for which data are not available are of obvious value for identification and structural diagnosis. In Fig. 3, $\nu_{C=O}$ for peroxides, per-esters, and per-acids [$\nu_{C=O}$ for acids are from Flett (*loc. cit.*) and unpublished work in this laboratory] are plotted against an arbitrary parameter (f) which is, where applicable, the mean of the four frequencies for peroxide and acid. Where all four frequencies are not known, the parameter f is chosen to fit the available frequencies to their corresponding curves. Experimentally measured points fall on the straight lines within 3 cm.$^{-1}$ in most cases, and this is not greatly in excess of experimental errors. The biggest discrepancies are in the monomers of maleic (1), phthalic (2), dichloroacetic (3), and trichloroacetic (4) acids, the numerals in parentheses referring to points in Fig. 4. These will be dealt with in Part II. Flett (*loc. cit.*) has shown that a smooth relationship exists between reaction rates (measured by Hammett's σ factors) and $\nu_{C=O}$ for a series of benzoic acids, and has suggested the use of such curves to forecast reaction rates from the more readily obtainable spectroscopic measurements. In Fig. 4, $\nu_{C=O}$ for dibenzoyl peroxides are plotted against σ, together with Flett's results (*loc. cit.*) for the corresponding acids. The agreement is comparable with Flett's, as is of course to be expected from the correlations of Fig. 3. Since the only simple physical relationship between carbonyl frequency and reaction rates can be in the force constants of bonds whose fission is involved in the reaction, such relationships between carbonyl frequency and reaction rates can only be expected for simple reaction mechanisms. For structural diagnosis the method is first to decide the type of carbonyl compound (*e.g.*, from the number of carbonyl absorptions) and the value of f obtained from

Fig. 3 then assists in the identification of the acyl radical. Alternatively, if the nature of the acyl radical is known, $\nu_{C=O}$ for the corresponding acid fixes f and thus determines the type of carbonyl compound.

Cooper (*loc. cit.*) and Swaine, Stockmeyer, and Clarke (*J. Amer. Chem. Soc.*, 1950, **72**, 5426) have shown that there is a direct relation between the rate of decomposition of the peroxide and σ in the case of substituted benzoyl peroxides. A similar relationship must exist between $\nu_{C=O}$ and decomposition rates for these compounds.

There is considerable theoretical interest in correlating the strength of the C=O, C–O, and O–O bonds in diacyl peroxides. Such correlations would facilitate the search for the elusive ν_{O-O} absorption, and, more important, enable O–O bond strengths to be calculated from spectroscopic data. A direct measurement of either the C–O or the O–O strength from vibrational frequency appears impossible, since probably neither frequency is characteristic (*i.e.*, unaffected by small mass effects). The only other vibration which is characteristic is ν_{O-H} of the corresponding acid monomer, the accurate measurement of which requires a higher resolution than that of a sodium chloride prism, and for which few values are available. Thompson (quoted by Flett, *loc. cit.*) gives monomeric ν_{O-H} values for five benzoic acids, which bear a smooth relationship to both $\nu_{C=O}$ and free energy of ionisation ($F_I = RT \ln K$, where K is the ionisation constant) of the corresponding acid.

A plot of carbonyl frequencies against F_I (Fig. 5) for a number of peroxides shows that aromatic peroxides fall on one curve, and aliphatic peroxides on another. (Ionisation data from "Int. Critical Tables," McGraw-Hill, N.Y., 1929, VI, p. 259 *et seq.*) This illustrates the fact that this completely empirical approach is only adequate within a class of similar compounds.

These correlations, *viz.*, between $\nu_{C=O}$ of peroxides and acids, and the strength of the hydroxyl bond as measured spectroscopically and by ionisation constants, show that a smooth relationship is to be expected between the strength of the O–O bond and $\nu_{C=O}$ within a series of similar compounds. In the case of the acid, a stronger carbonyl (*i.e.*, higher frequency) corresponds to a weaker O–H bond, and at first this may suggest that it corresponds to a weaker O–O bond. Walsh (*J.*, 1948, 331) has discussed the effect of electron shifts towards the peroxide bond, and considered that initially such a shift would strengthen the bond, but that still greater shifts towards the peroxide bond might result in a weakening of the bond. The matter is still further complicated by the fact that a steric effect which results in a twisting of the bond out of its most stable skew form would result in a weakening of the bond owing to repulsion of the lone-pairs. Thus, although in general a *smooth* relationship is expected betweeen carbonyl bond strengths and peroxide bond strengths, its form and even its sense cannot be readily predicted.

It is intended to examine the theoretical basis and limitations of these correlations in a second paper, which will also cover a larger range of carbonyl compounds.

The author thanks Dr. W. Cooper for providing most of the compounds used, and Mr. C. E. Kendall and Dr. Cooper for helpful discussion. He is indebted to Mr. G. R. Bates for assistance in the experimental work, and to the Dunlop Rubber Company for permission to publish it.

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543. *Experiments on the Synthesis of* Bz-*Substituted Indoles and Tryptophans. Part II.* Attempted Syntheses of* Bz-*Nitro-indoles and -tryptophans. The Synthesis of* 5-*Ethoxytryptophan and of* 4 : 5- *and* 6 : 7-*Benzotryptophan.*

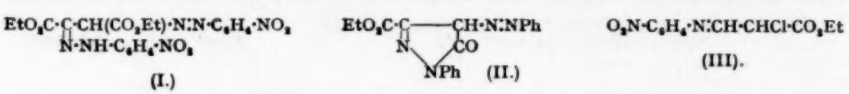
By H. N. Rydon and S. Siddappa.

Unsuccessful attempts to synthesise *Bz*-nitro-indoles and -tryptophans by a variety of methods are described. 5-Ethoxytryptophan, and 4 : 5- and 6 : 7-benzotryptophan have been synthesised, by conventional methods, for test as bacterial inhibitors.

For the continuation of the study of the relation between structure and antibacterial activity of substituted indoles and tryptophans (Fildes and Rydon, *Brit. J. Exp. Path.*, 1947, **28**, 211; Rydon, *J.*, 1948, 705 *; Rydon and Long, *Nature*, 1949, **164**, 575) the nitroindoles and nitrotryptophans are desirable compounds, both for their intrinsic interest as substances containing a powerfully electron-attracting group and as intermediates for the preparation of other *Bz*-substituted indoles and tryptophans. We have, accordingly, examined several possible methods for the preparation of these substances; although we did not achieve our object it is desirable to report our findings at this stage in view of the termination of our collaboration on this subject.

Comparatively few *Bz*-nitroindoles are described in the literature. Majima and Kotake (*Ber.*, 1930, **63**, 2237) obtained 6-nitroindole by nitration of 3-carbethoxyindole, followed by hydrolysis and decarboxylation, and an unorientated nitroindole-3-aldehyde by direct nitration of the well-known indole-3-aldehyde; Bauer and Strauss (*Ber.*, 1932, **65**, 308) prepared 2 : 3-dimethyl-5-nitroindole by nitration of 2 : 3-dimethylindole, and von Walther and Clemen (*J. pr. Chem.*, 1900, **61**, 266) obtained an unorientated 2-methyldinitroindole by direct nitration of skatole. Such direct nitration methods were insufficiently flexible for our purpose, since we required all four *Bz*-nitroindoles.

The Fischer synthesis seemed more promising; various 2 : 3-disubstituted *Bz*-nitroindoles have been prepared in this way from the *o*-, *m*-, and *p*-nitrophenylhydrazones of ketones (Bauer and Strauss, *loc. cit.*; Schofield and Theobald, *J.*, 1949, 796; 1950, 1505), and Hughes, Lions, and Ritchie (*J. Proc. Roy. Soc., N.S.W.*, 1939, **72**, 309) describe the synthesis of 7-nitroindole by Fischer cyclisation of ethyl pyruvate *o*-nitrophenylhydrazone, followed by hydrolysis and decarboxylation. We have been unable to confirm the findings of the Australian workers, ethyl pyruvate *o*-nitrophenylhydrazone failing, in our hands, to cyclise under a variety of conditions; with aqueous acids the only product was pyruvic acid *o*-nitrophenylhydrazone, m. p. 222°, which appears to be the substance described by Hughes, Lions, and Ritchie (*loc. cit.*) as 7-nitroindole-2-carboxylic acid (m. p. 231°), and under non-aqueous conditions we obtained a substance isomeric with, and possibly a stereoisomeride of, the starting material. The



analytical figures quoted by Hughes, Lions, and Ritchie (*loc. cit.*) for 7-nitroindole-2-carboxylic acid and for 7-nitroindole are unsatisfactory and it seems doubtful whether they in fact obtained these compounds.† Similarly, attempts to cyclise the *m*- and *p*-nitrophenylhydrazones of ethyl pyruvate gave only the derivatives of pyruvic acid. Attempts to modify the structural conditions by replacing ethyl pyruvate by ethyl acetosuccinate were rendered abortive since this substance reacts with two molecules of diazotised base in the Japp–Klingemann reaction, giving (I) with diazotised *p*-nitroaniline and (II) with diazotised aniline. Similar lack of success attended attempts to prepare the *Bz*-nitrotrytophans directly by cyclisation of the nitrophenylhydrazones of the Michael adduct (XII) from acraldehyde and acetamidomalonic ester (cf. Warner and Moe, *J. Amer. Chem. Soc.*, 1948, **70**, 2763, 2765). These consistent failures to

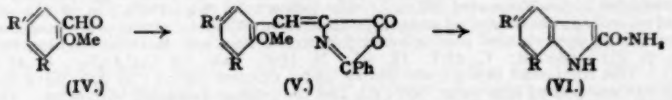
* *J.*, 1948, 705 is regarded as Part I of this series.

† In a personal communication Dr. Hughes informs us that he and his colleagues are in agreement with this conclusion.

cyclise nitrophenylhydrazones are doubtless to be ascribed to the deactivating effect of the nitro-group, although this cannot be the sole reason in view of the successful cyclisations of ketone nitrophenylhydrazones recorded by other workers (Bauer and Strauss, *loc. cit.*; Schofield and Theobald, *locc. cit.*).

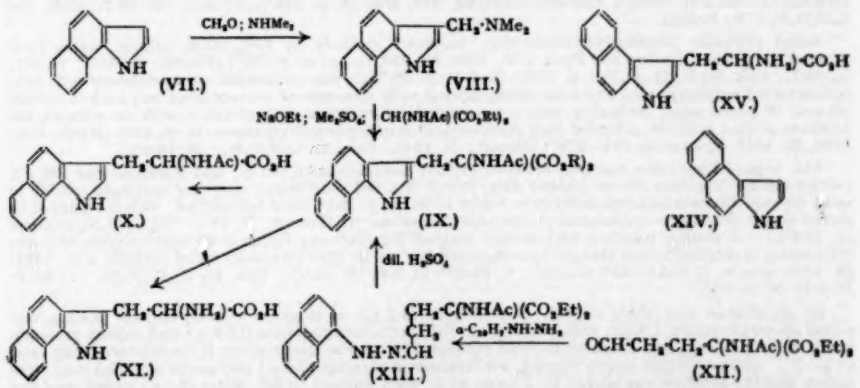
We next turned our attention to Mentzer's alleged synthesis of 2-carbethoxyindoles by the action of aromatic amines on ethyl formylchloroacetate (*Compt. rend.*, 1946, **222**, 1178). With the three nitroanilines the only products were the anils (III), and reinvestigation of two of the cases described by Mentzer, *viz.*, reaction with aniline and *p*-anisidine, showed that here, too, anils were the sole products (cf. the papers of Smith, *J.*, 1950, 1637; Robinson and Saxton, *ibid.*, p. 3136, and Taylor, *ibid.*, p. 3345, which appeared after the completion of our work).

Hill and Robinson (*J.*, 1933, 486) prepared 5 : 7-dinitroindole-2-carboxyamide (dinitrostrycholamide) (VI; R = R′ = NO_2) by the action of alcoholic ammonia on the oxazolone (V; R = R′ = NO_2) obtained from 2-methoxy-3 : 5-dinitrobenzaldehyde (IV; R = R′ = NO_2), and we attempted to prepare the 5-nitro-compound (VI; R = H, R′ = NO_2) similarly : 2-methoxy-5-nitrobenzaldehyde (IV; R = H, R′ = NO_2), prepared with some difficulty from 5-nitrosalicylaldehyde, condensed normally with hippuric acid to give the oxazolone (V; R = H, R′ = NO_2) but this did not yield any of the required indole derivative with alcoholic ammonia. In this case it appears that a single nitro-group insufficiently activates the methoxyl group.



Special interest attaches to the benzindoles and benzotryptophans as bacterial inhibitors in view of the finding (Albert, Rubbo, Goldacre, Davey, and Stone, *Brit. J. Exp. Path.*, 1945, **26**, 160) that, in the acridine series, inhibitory activity is considerably modified by changes in the "flat" area of the inhibitor molecule. We have, accordingly, prepared the two most readily accessible benzindoles and benzotryptophans with a view to determining whether such an effect also operates in the indole–tryptophan series.

6 : 7-Benzindole (VII) was first prepared by Schlieper (*Annalen*, 1887, **239**, 229) by the Fischer cyclisation of pyruvic acid α-naphthylhydrazone with zinc chloride, followed by decarboxylation of the resulting 6 : 7-benzindole-2-carboxylic acid; the structure of the product was confirmed by two unambiguous syntheses (Pschorr and Kuhtz, *Ber.*, 1905, **38**, 217; Mayer and Oppenheimer, *ibid.*, 1918, **51**, 510, 1239). 6 : 7-Benzindole (VII), prepared in increased yield by ethanolic sulphuric acid cyclisation of ethyl pyruvate α-naphthylhydrazone, was converted into 6 : 7-benzotryptophan (XI) by the gramine method (Snyder and Smith, *J. Amer. Chem. Soc.*, 1944, **66**, 350; Albertson, Archer, and Suter, *ibid.*, p. 500; 1945, **67**, 36; cf. Rydon, *J.*, 1948, 705) thus :



6 : 7-Benzotryptophan (XI) was also synthesised by the elegant method of Warner and Moe (*J. Amer. Chem. Soc.*, 1948, **70**, 2763, 2765), cyclisation of the α-naphthylhydrazone (XIII) of

the Michael adduct (XII) from acraldehyde and acetamidomalonic ester giving an excellent yield of the intermediate (IX), identical with that obtained by the gramine method.

Precisely analogous methods were used for the synthesis of 4 : 5-benzotryptophan (XV) from 4 : 5-benzindole (XIV); the latter was first prepared by Schlieper (*Annalen*, 1886, **236**, 177) by Fischer cyclisation of ethyl pyruvate β-naphthylhydrazone, a process which we have considerably improved; its structure rests on its non-identity with 5 : 6-benzindole, the other possible product of the Fischer cyclisation, which has been prepared by another route (G.P. 516,675).

We record also (p. 2466) the synthesis of 5-ethoxyindole (cf. Hoshino and Kotake, *Annalen*, 1935, **516**, 76; Deulofeu, *Rev. brasil. Chim.*, 1938, **5**, 270) by the Fischer method from ethyl pyruvate *p*-ethoxyphenylhydrazone and its conversion into 5-ethoxytryptophan by the gramine procedure; in this case Warner and Moe's method (*loc. cit.*) was less satisfactory.

Biological tests on the indoles and tryptophans described in this paper will be reported elsewhere in due course.

Experimental.

A. *Attempted Syntheses of* Bz-*Nitrotryptophans.*—(i) *By the Fischer method.* Ethyl pyruvate *o*-nitrophenylhydrazone (10 g.; m. p. 114°; Hughes, Lions, and Ritchie, *loc. cit.*, give m. p. 106°) (Found: C, 52·65; H, 5·2; N, 16·95. Calc. for $C_{11}H_{13}O_4N_3$: C, 52·6; H, 5·2; N, 16·7%) was refluxed for 1 hour with concentrated hydrochloric acid (90 c.c.). The yellow solid (9 g., 100%; m. p. 218—220°), which crystallised on cooling, was filtered off and recrystallised from ethanol, forming yellow needles, m. p. 220°, which were clearly pyruvic acid *o*-nitrophenylhydrazone (Ciusa and Rastelli, *Gazzetta*, 1922, **52**, 121, record m. p. 211°) (Found: C, 48·7; H, 4·3; N, 18·8. Calc. for $C_9H_9O_4N_3$: C, 48·4; H, 4·0; N, 18·8%). This compound (8·5 g.) was heated under glycerol (90 c.c.) to 220° for 5 minutes; the cooled mixture was poured into water (500 c.c.), and the product extracted with ether. Distillation of the dried extract yielded only *o*-nitroaniline (3·5 g., 66%), orange rods (from light petroleum), m. p. and mixed m. p. 70°; no trace of nitroindole could be detected in the reaction product. Ethyl pyruvate *o*-nitrophenylhydrazone gave only the pyruvic acid derivative with a solution of zinc chloride in boiling concentrated hydrochloric acid, but, when it was heated (2 g.) with acetic acid (10 c.c.), containing concentrated sulphuric acid (1 c.c.) to 80° and poured into water, a yellow solid separated which crystallised from ethanol in yellow needles, m. p. 97°; this *substance* (Found: C, 52·3; H, 5·2; N, 16·9. $C_{11}H_{13}O_4N_3$ requires C, 52·6; H, 5·2; N, 16·7%) is isomeric with, and possibly a stereoisomeride of, ethyl pyruvate *o*-nitrophenylhydrazone; it was also formed by the action of cold concentrated sulphuric acid and yielded pyruvic acid *o*-nitrophenylhydrazone, m. p. and mixed m. p. 220°, on hydrolysis with concentrated hydrochloric acid.

Ice-cold 50% potassium hydroxide solution (50 c.c.) was added to a stirred solution of ethyl methylacetoacetate (20·5 g.) in ethanol (150 c.c.) at 0°. Ice-water (300 c.c.) was added, followed immediately by a diazonium solution from *m*-nitroaniline (20 g.), concentrated hydrochloric acid (60 c.c.), sodium nitrite (10·5 g.), and water (90 c.c.). The crystalline solid, which separated after a further 15 minutes' stirring, was recrystallised from hot methanol; *ethyl pyruvate* m-*nitrophenylhydrazone* (30 g., 84%) forms orange needles, m. p. 150° (Found: C, 53·3; H, 5·3; N, 16·9. $C_{11}H_{13}O_4N_3$ requires C, 52·6; H, 5·2; N, 16·7%). This substance was recovered unchanged after being heated on the steam-bath for 2 hours with acetic acid (5 vols.) and concentrated sulphuric acid (0·5 vol.); refluxing for 1 hour with concentrated hydrochloric acid (5 vols.) yielded pyruvic acid *m*-nitrophenylhydrazone, m. p. 220° (from ethanol) (Ciusa and Musajo, *Gazzetta*, 1930, **60**, 486, give m. p. 226°) (Found: N, 19·1. Calc. for $C_9H_9O_4N_3$: N, 18·8%).

Ethyl pyruvate *p*-nitrophenylhydrazone, prepared similarly in 78% yield, orange plates from ethanol, m. p. 184° (Quilico and Freri, *ibid.*, 1929, **59**, 930, record m. p. 187°) (Found: C, 52·5; H, 5·3; N, 16·7. Calc. for $C_{11}H_{13}O_4N_3$: C, 52·6; H, 5·2; N, 16·7%), was unchanged after treatment with cold concentrated sulphuric acid and after being heated with mixtures of concentrated sulphuric acid and ethanol or acetic acid; refluxing with concentrated hydrochloric acid (5 vols.), with or without the addition of zinc chloride, afforded only pyruvic acid *p*-nitrophenylhydrazone, m. p. 225° (Hyde, *Ber.*, 1899, **32**, 1815, gives m. p. 219—220°) (Found: N, 19·0. Calc. for $C_9H_9O_4N_3$: N, 18·8%).

The Japp–Klingemann reaction between diethyl acetosuccinate (30 g.) and *p*-nitroaniline (20 g.), carried out as described above, yielded 25 g. (38%) of a red solid which was very sparingly soluble in most organic solvents and separated in powdery form, m. p. 235°, from hot dioxan; this is regarded as *diethyl* p-*nitrobenzeneazo-oxaloacetate* p-*nitrophenylhydrazone* (I) (Found: N, 18·5. $C_{20}H_{20}O_9N_6$ requires N, 17·8%). A similar reaction with aniline yielded 3-carbethoxy-1-phenyl-4-phenylazopyrazol-5-one (II), which crystallised from ethanol in orange needles, m. p. 153° (Wislicenus and Scheidt, *Ber.*, 1891, **24**, 4210, give m. p. 152—154°) (Found: C, 64·25; H, 4·9; N, 16·75. Calc. for $C_{18}H_{16}O_3N_4$: C, 64·3; H, 4·7; N, 16·7%).

(ii) *By Warner and Moe's method.* Acraldehyde (3·2 g.), in magnesium-dried ethanol (5 c.c.), was added dropwise during 1 hour, with stirring, to ethyl acetamidomalonate (10·9 g.) and sodium ethoxide (from sodium, 0·05 g., and magnesium-dried ethanol, 25 c.c.), the temperature of the mixture being kept at 3—7°. After a further hour's stirring, *o*-nitrophenylhydrazine (7·5 g.) and acetic acid (1·5 c.c.) were added, and the mixture was stirred for 2 hours at 3°, then warmed to 50°, water (2 c.c.) added, and the mixture kept overnight. Dilution with water and ice-cooling precipitated a yellow oil which solidified after a few hours (12 g., 59%); crystallisation from methanol yielded *γ-acetamido-γγ-dicarbethoxy*-n-*butaldehyde* o-*nitrophenylhydrazone* as yellow needles, m. p. 40° (Found: N, 14·1. $C_{18}H_{24}O_7N_4$ requires

N, 13·7%). The corresponding m-*nitrophenylhydrazone* (63% yield), orange prisms (from aqueous acetone), m. p. 150° (Found: C, 53·4; H, 6·1; N, 13·6. $C_{18}H_{24}O_7N_4$ requires C, 52·9; H, 5·9; N, 13·7%), and p-*nitrophenylhydrazone* (73% yield), yellow plates [from benzene–light petroleum (b. p. 40—60°)], m. p. 130° (Found: C, 52·9; H, 6·0; N, 14·0%), were prepared similarly. The *p*-nitrophenylhydrazone gave only tarry material on refluxing with 5% (v/v) sulphuric acid for 4 hours, but the *m*-nitrophenylhydrazone was unaffected by this treatment. The *o*-nitrophenylhydrazone (2·0 g.) was refluxed with 5% (v/v) sulphuric acid (12 c.c.) for 3 hours; a crystalline solid (0·5 g.) separated which crystallised from aqueous ethanol in pale yellow needles, m. p. 100—101°, which proved to be the *o-nitrophenylhydrazine* salt of 1-hydroxybenzotriazole (Found: N, 29·4. $C_{18}H_{12}O_5N_8$ requires N, 29·2%).

(iii) *By Mentzer's method.* *p*-Nitroaniline (2·6 g.) and ethyl formylchloroacetate (Wislicenus, *Ber.*, 1910, **43**, 3530) (4·6 g.) were refluxed for 5 minutes; the entire mixture solidified, and digestion with ethanol gave a yellow solid (4 g., 79%); *ethyl α-chloro-β-(p-nitrophenylimino)propionate* (III) crystallised from ethanol in yellow needles, m. p. 215° (Found: C, 48·9; H, 4·2; N, 10·4. $C_{11}H_{11}O_4N_2Cl$ requires C, 48·8; H, 4·1; N, 10·35%). The same product was obtained when the heating was carried out for 90 minutes at 130° and when the reactants were heated for 4 hours in acetic acid containing a little morpholine.

The following were prepared similarly from the appropriate amines: *Ethyl α-chloro-β-o-nitrophenyliminopropionate*, yellow plates (from methanol), m. p. 101° (Found: C, 48·7; H, 4·1; N, 10·0%); *ethyl α-chloro-β-m-nitrophenyliminopropionate*, yellow plates (from methanol), m. p. 137° (Found: C, 48·75; H, 4·35; N, 10·25%); ethyl α-chloro-β-phenyliminopropionate, plates (from methanol), m. p. 82° (Smith, *loc. cit.*, gives m. p. 82°) (Found: C, 58·5; H, 5·5; N, 6·1; Cl, 15·85. Calc. for $C_{11}H_{12}O_2NCl$: C, 58·5; H, 5·3; N, 6·2; Cl, 15·7%); ethyl α-chloro-β-*p*-methoxyphenyliminopropionate, prisms (from aqueous methanol), m. p. 97° (Smith, *loc. cit.*, gives m. p. 96—96·5°) (Found: C, 56·85; H, 5·55; N, 5·6; Cl, 14·35. Calc. for $C_{12}H_{14}O_3NCl$: C, 56·4; H, 5·5; N, 5·5; Cl, 13·9%).

(iv) *By Hill and Robinson's method.* 5-Nitrosalicylaldehyde (Miller, *Ber.*, 1887, **20**, 1928) (4·2 g.) was mixed with methyl iodide (12 g.), and dry silver oxide (8·7 g.) added in small quantities under reflux; after the initial reaction had abated, the mixture was heated on the steam-bath for 1 hour, cooled, diluted with ether, filtered, and evaporated. Crystallisation from methanol gave 2-methoxy-5-nitrobenzaldehyde (IV; R = H, R′ = NO_2) as yellow needles, m. p. 80° (lit., m. p. 89°), sufficiently pure for the next stage. This aldehyde (1·8 g.), anhydrous sodium acetate (0·54 g.), hippuric acid (1·8 g.), and acetic anhydride (10 c.c.) were gently refluxed for 1 hour. The yellow solid which separated on cooling was filtered off and washed with water and ethanol (yield, 1·9 g., 59%; m. p. 225—230°); crystallisation from acetic acid gave 4-(*2-methoxy-5-nitrobenzylidene*)-*2-phenyloxazol-5-one* (V; R = H, R′ = NO_2) as pale yellow needles, m. p. 253—254° (Found: C, 62·5; H, 3·9; N, 8·9. $C_{17}H_{12}O_5N_2$ requires C, 63·0; H, 3·7; N, 8·6%).

B. *Synthesis of the Benzotryptophans.*—**6 : 7-***Benzindole*. Ethyl pyruvate α-naphthylhydrazone (Hughes and Lions, *J. Proc. Roy. Soc., N.S.W.*, 1937, **71**, 475) (20 g.) was refluxed for 24 hours with anhydrous ethanol (180 c.c.) and concentrated sulphuric acid (10 c.c.). The mixture was poured on crushed ice (600 g.) and the product, which crystallised after some hours, was recrystallised from methanol. This ester (17 g., 91%; m. p. 160°; Schlieper, *Annalen*, 1887, **239**, 229, gives m. p. 170°) was refluxed for 3 hours with potassium hydroxide (20 g.) in methanol (100 c.c.); after removal of most of the methanol the residue was acidified, and the precipitated 6 : 7-benzindole-2-carboxylic acid filtered off and washed with water and a little methanol; m. p. 204° (Schlieper, *loc. cit.*, gives m. p. 204—205°); yield 14 g. (93%).

6 : 7-Benzindole-2-carboxylic acid (5 g.) was heated to 210—220° (metal-bath); decarboxylation was complete in 10 minutes and the cooled product was sublimed in a high vacuum at 130—140° (2·6 g., 66%; m. p. 179—180°). Crystallisation from aqueous methanol gave 6 : 7-benzindole (VII) in flakes, m. p. 180° (Schlieper, *loc. cit.*, gives m. p. 179—180°) (Found: C, 85·9; H, 5·4; N, 8·6. Calc. for $C_{13}H_9N$: C, 86·2; H, 5·4; N, 8·4%).

6 : 7-*Benzotryptophan*. Glacial acetic acid (15 c.c.) was added slowly to aqueous dimethylamine (33%; 15 c.c.) with cooling sufficient to keep the temperature below 5°; aqueous formaldehyde (40%; 7·5 c.c.) was added, and one-eighth of the resulting mixture added to 6 : 7-benzindole (2 g.). A clear solution was obtained after 4 hours; this was poured into 2N-sodium hydroxide (75 c.c.) and kept at 0° overnight. The resulting *3-dimethylaminomethyl-6 : 7-benzindole* (VIII) crystallised from aqueous methanol in prisms, m. p. 172° (1·7 g., 63%) (Found: C, 80·4; H, 7·2; N, 12·4. $C_{15}H_{16}N_2$ requires C, 80·4; H, 7·1; N, 12·5%). This benzogramine (1·12 g.) was added to acetamidomalonic ester (1·08 g.), dissolved in sodium ethoxide (from sodium, 0·12 g., and magnesium-dried ethanol, 15 c.c.), and treated with methyl sulphate (1·36 g.). The mixture was kept at room temperature for 24 hours and then poured into water (100 c.c.); after being kept overnight at 0°, the *ethyl α-acetamido-α-(6 : 7-benz-3-indolylmethyl)malonate* (IX; R = Et) (1·9 g., 96%; m. p. 166—167°) was filtered off; the pure compound crystallises from aqueous methanol in prisms, m. p. 171° (Found: C, 66·5; H, 6·1; N, 7·0. $C_{22}H_{24}O_5N_2$ requires C, 66·7; H, 6·0; N, 7·1%). The crude condensation product (3·5 g.) was refluxed for 4 hours with sodium hydroxide (5 g.) in water (15 c.c.) and ethanol (25 c.c.). After removal of the ethanol, the residue was dissolved in water (100 c.c.) and acidified; ether-extraction, followed by evaporation of the dried extract, yielded *α-acetamido-α-(6 : 7-benz-3-indolylmethyl)malonic acid* (IX; R = H) (1·9 g., 62%), which crystallised from ethyl acetate–petroleum (b. p. 60—80°) in prisms, m. p. 180° (Found: N, 7·9. $C_{18}H_{16}O_5N_2$ requires N, 8·2%). This acid (1·7 g.) was heated to 210° in a current of nitrogen for 30 minutes; the product, *N-acetyl-6 : 7-benzotryptophan* (X), crystallised from aqueous methanol in prisms, m. p. 235° (Found: C, 68·5; H, 5·6; N, 9·3. $C_{17}H_{14}O_3N_2$ requires C, 68·9; H, 5·4; N, 9·4%), but could not satisfactorily be hydrolysed to the parent amino-acid. Accordingly, the crude condensation product (IX; R = Et) (1 g.) was hydrolysed, as described above,

to (IX; R = H), and the crude product refluxed for 3 hours with water and concentrated hydrochloric acid (10 c.c.; 1 : 1). The mixture was cooled, filtered from a little tar, and brought to pH 5—6; the solid (0·5 g., 75%), which separated overnight, was collected by filtration and reprecipitated from dilute alkali, after use of charcoal, by adjustment of the pH to 5—6 with acetic acid. **6 : 7-*Benzotryptophan*** (XI), so obtained, was a pale yellow solid, m. p. 303° (placed in bath at 280°) (Found : C, 71·0; H, 5·9; N, 10·7. $C_{13}H_{14}O_2N_2$ requires C, 70·8; H, 5·5; N, 11·0%), which was very sparingly soluble in water, giving a positive ninhydrin reaction, a green colour in the Hopkins–Cole glyoxylic reaction (Cole, " Practical Physiological Chemistry," Hefter, Cambridge, 5th edn., 1919, p. 39), and a green turning to blue in Cole's formaldehyde reaction (*op. cit.*, 9th edition, 1933, p. 80).

Alternatively, (IX; R = Et) was prepared by the following much more economical procedure : Ethyl acetamidomalonate (10·9 g.) was dissolved in sodium ethoxide (from sodium 0·05 g., and absolute ethanol, 25 c.c.), and acraldehyde (3·2 g.), in absolute ethanol (5 c.c.), was added dropwise during 1 hour, with stirring and cooling to keep the mixture at 3—7°. After a further 3 hours' stirring, acetic acid (1·5 c.c.) and α-naphthylhydrazine (7·5 g.) were added, and the mixture warmed to 50° and then kept at room temperature overnight after the addition of water (2 c.c.). Addition of more water precipitated a semi-solid mass (XIII), which was refluxed for 4 hours with 5% (v/v) sulphuric acid (105 c.c.). On cooling, a brown solid separated which, after two crystallisations from aqueous ethanol, yielded the ester (IX; R = Et) (8 g., 54%), m. p. 171°, not depressed on admixture with material prepared by the gramine method.

4 : 5-*Benzindole*. Ethyl pyruvate β-naphthylhydrazone (Hughes and Lions, *loc. cit.*) (25 g.) was refluxed for 20 hours with ethanol (150 c.c.) and concentrated sulphuric acid (15 c.c.). The mixture was poured on crushed ice (500 g.), and the crude ethyl 4 : 5-benzindole-2-carboxylate filtered off; a specimen recrystallised once from light petroleum (b. p. 60—80°) and once from aqueous methanol had m. p. 162° (Schlieper, *Annalen*, 1886, **236**, 177, gives m. p. 161°) (Found : C, 75·3; H, 5·4; N, 6·0. Calc. for $C_{15}H_{13}O_2N$: C, 75·3; H, 5·4; N, 5·9%). The bulk of the crude ester was refluxed for 3 hours with sodium hydroxide (20 g.) in methanol (150 c.c.); acidification, followed by redissolution in aqueous ammonia, decolorisation (charcoal), and reprecipitation with dilute hydrochloric acid, yielded 4 : 5-benzindole-2-carboxylic acid (15 g.; 80%), m. p. 226°, sufficiently pure for decarboxylation; the pure acid crystallised from acetic acid in flakes, m. p. 246° (Schlieper, *loc. cit.*, gives m. p. 226°) (Found : C, 73·9; H, 4·5; N, 6·5. Calc. for $C_{13}H_9O_2N$: C, 73·9; H, 4·3; N, 6·6%). The crude acid (10 g.) was suspended in dry ether (150 c.c.) and cooled in a freezing mixture while an excess of liquid ammonia was added dropwise; after being kept for 30 minutes, the ammonium salt was filtered off, dried, and heated gently over a free flame until decarboxylation was complete; distillation gave 3·9 g. (49%) of 4 : 5-benzindole (XIV), b. p. 144°/0·5 mm. The picrate, prepared in ethanol, crystallised from benzene in red needles, m. p. 202° (Found : C, 55·1; H, 3·2. Calc. for $C_{18}H_{12}O_7N_4$: C, 54·5; H, 3·0%).

4 : 5-*Benzotryptophan*. *Ethyl α-acetamido-α-(4 : 5-benz-3-indolylmethyl)malonate* prepared, as described above for the 6 : 7-isomer, from ethyl acetamidomalonate, acraldehyde, and β-naphthylhydrazine in 67% yield, crystallised from aqueous methanol in plates, m. p. 192° (Found : C, 66·1; H, 6·3; N, 7·4%). This ester (2 g.) was hydrolysed and decarboxylated, as described above for the 6 : 7-analogue, yielding crude 4 : 5-benzotryptophan (1 g., 78%); reprecipitation from dilute alkali by adjustment to pH 5—6 gave **4 : 5-*benzotryptophan*** (XV), as a yellow solid, m. p. 275° (in bath at 260°) (Found : C, 71·2; H, 6·1; N, 10·9, 11·1%), almost insoluble even in hot water and giving an emerald-green colour in the Hopkins–Cole glyoxylic reaction and a green turning to greenish-blue in the Cole formaldehyde reaction.

In a less advantageous procedure, freshly distilled 4 : 5-benzindole (3·5 g.) was treated, as described for the 6 : 7-isomer, with dimethylamine and formaldehyde; the crude product (4·6 g., 97%) was crystallised from aqueous methanol, yielding ***3-dimethylaminomethyl-4 : 5-benzindole*** in plates, m. p. 162° (Found : C, 80·4; H, 7·4; N, 12·6%). Condensation of this gramine (1·12 g.) with acetamidomalonic ester, as described for the 6 : 7-isomer, gave 1·7 g. (86%) of ethyl α-acetamido-α-(4 : 5-benz-3-indolylmethyl)malonate, m. p. 192° not depressed on admixture with material prepared by the alternative route.

C. *Synthesis of* **5-*Ethoxytryptophan*.**—Ethyl pyruvate *p*-ethoxyphenylhydrazone (Jacobson, *Annalen*, 1922, **427**, 142) (60 g.) was refluxed for 20 hours with absolute ethanol (300 c.c.) containing concentrated sulphuric acid (5 c.c.); the dark oil, which separated on pouring on ice (800 g.), solidified when rubbed and was crystallised from methanol; it had m. p. 145—155°. This crude ethyl 5-ethoxyindole-2-carboxylate (15 g., 27%) was refluxed for 3 hours with potassium hydroxide (15 g.) in methanol (100 c.c.); removal of the methanol and acidification yielded 5-ethoxyindole-2-carboxylic acid which, crystallised from aqueous methanol, had m. p. 202—203° (Hoshino and Kotake, *Annalen*, 1935, **516**, 76, record m. p. 203—204°); the yield was 10 g. (96%). This acid (6·5 g.) was heated to 220—230° for 10 minutes; distillation of the product yielded 5-ethoxyindole (2 g., 46%), b. p. 124°/0·5 mm. This was treated as usual with dimethylamine and formaldehyde; the indole dissolved within 10 minutes, and the reaction mixture was poured into 2N-sodium hydroxide (75 c.c.) after being kept for 4 hours at room temperature. ***3-Dimethylaminomethyl-5-ethoxyindole*** (1·6 g., 59%) was collected after being kept at 0° overnight and recrystallised from aqueous ethanol in cubes, m. p. 140° (Found : C, 71·7; H, 8·5; N, 12·6. $C_{13}H_{18}ON_2$ requires C, 71·6; H, 8·2; N, 12·8%). Condensation of this 5-ethoxygramine (2 g.) with ethyl acetamidomalonate in the usual manner afforded *ethyl α-acetamido-α-(5-ethoxy-3-indolylmethyl)malonate* (3·5 g., 98%) which crystallised from aqueous methanol in needles, m. p. 169° (Found : C, 61·6; H, 6·8; N, 7·2. $C_{20}H_{24}O_6N_2$ requires C, 61·5; H, 6·7; N, 7·2%). The crude ester (2 g.) was refluxed for 3 hours with sodium hydroxide (2 g.) in water (10 c.c.) and ethanol (12 c.c.); the alcohol was removed under reduced pressure, and the residue taken up in water, cooled to 0°, acidified, and extracted with ether. Evaporation of the dried extract and crystallisation of the residue from ethyl acetate–light petroleum (b. p. 60—80°) yielded *α-acetamido-α-(5-ethoxy-3-indolylmethyl)malonic acid* (1·2 g., 70%) in needles, m. p.

147° (Found: N, 7·6. $C_{16}H_{16}O_6N_2$ requires N, 8·4%). The dry acid (1 g.) was heated to 165—170° for 30 minutes in a current of nitrogen; crystallisation of the residue from hot water yielded *1-acetyl-5-ethoxytryptophan* (0·7 g., 80%) in needles, m. p. 195° (Found: C, 61·6; H, 6·5; N, 9·1. $C_{15}H_{18}O_4N_2$ requires C, 62·1; H, 6·2; N, 9·6%). This acetyl compound (1·3 g.) was heated at 100° for 24 hours, with exclusion of atmospheric carbon dioxide, with barium hydroxide (3·8 g.) in water (15 c.c.); boiling water (80 c.c.) was added, and barium ions removed by the addition of N-sulphuric acid. After separation of the barium sulphate, the solution was concentrated to 20 c.c. under reduced pressure; *5-ethoxytryptophan* (0·5 g., 45%) gradually crystallised and was recrystallised from 50% aqueous ethanol, forming colourless needles, m. p. 248° (in bath at 230°) (Found: C, 62·5; H, 7·1; N, 11·2. $C_{13}H_{16}O_3N_2$ requires C, 62·9; H, 6·4; N, 11·3%), giving a blue colour in the Hopkins–Cole glyoxylic reaction and a blue turning to purple in Cole's formaldehyde reaction.

5-Ethoxygramine (1 g.) was refluxed for 90 minutes, under dry nitrogen, with powdered sodium hydroxide (0·07 g.) and formamidomalonic ester (1·2 g.) in dry toluene (5 c.c.). Light petroleum (b. p. 40—60°) was added to the product, cooled in ice, and the solid crystallised from aqueous methanol, yielding *ethyl α-(5-ethoxy-3-indolylmethyl)-α-formamidomalonate* (0·5 g., 30%) as needles, m. p. 153° (Found: C, 60·8; H, 6·5; N, 7·6. $C_{19}H_{24}O_6N_2$ requires C, 60·6; H, 6·4; N, 7·45%).

Ethyl pyruvate o-ethoxyphenylhydrazone, yellow plates (from aqueous methanol), m. p. 87° (Found: C, 62·4; H, 7·2; N, 11·2. $C_{13}H_{18}O_3N_2$ requires C, 62·4; H, 7·2; N, 11·2%), and the *p-methoxyphenylhydrazone*, yellow rods (from aqueous methanol), m. p. 97° (Found: C, 59·4; H, 6·5; N, 11·3. $C_{12}H_{16}O_3N_2$ requires C, 61·0; H, 6·8; N, 11·9%), were prepared in 55% yield by the Japp–Klingemann method; the *p*-methoxy-compound was converted, essentially as described for the *p*-ethoxy-compound, into 5-methoxyindole, m. p. 55° (Blaikie and Perkin, *J.*, 1924, **125**, 296, record m. p. 55°), in 47% overall yield.

We are grateful to the State Government of Mysore for a Scholarship which enabled one of us (S. S.) to take part in this work. Analyses were carried out in the microanalytical laboratory of this Department (Mr. F. H. Oliver).

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544. *The Synthesis of Thyroxine and Related Substances. Part VIII.* The Preparation of Some Halogeno- and Nitro-diphenyl Ethers.*

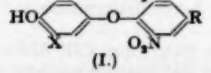
By J. C. CLAYTON, G. F. H. GREEN, and B. A. HEMS.

A number of dinitrodiphenyl ethers and their halogen derivatives have been prepared by methods described in the earlier papers of the series. The preparation of 3 : 5 : 3′ : 5′-tetranitro-L- and -DL-thyronine is described.

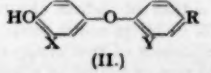
IN Part I of this series (Borrows, Clayton, and Hems *J.*, 1949, S 185) we stated that we hoped to prepare analogues of thyroxine, which might show antithyroid activity. At present the drugs employed in the treatment of hyperthyroidism are sulphur-containing compounds; they bear no structural relation to thyroxine and act by preventing the formation of the hormone, whereas chemical analogues might be expected to inhibit its peripheral effects.

Some analogues of thyroxine have already been prepared by other workers. Thus, Harington (*Biochem. J.*, 1948, **43**, 434) prepared the sulphide analogue; Woolley (*J. Biol. Chem.*, 1946, **164**, 11) prepared a few aliphatic ethers of *N*-acetyl-3 : 5-di-iodotyrosine; Frieden and Winzler (*ibid.*, 1949, **179**, 423) prepared 4-benzyloxy-3 : 5-di-iodobenzoic acid and several similar compounds. Many of these were found to have antithyroid activity when tested on tadpoles, but recent work (Maclagan and Sheahan, *J. Endocrinol.*, 1950, **6**, 456) has shown them to be much less effective in mice. Tetrahalogenated thyronines prepared by Shuegraf (*Helv. Chim. Acta*, 1929, **12**, 405), in which chlorine or bromine replaced the iodine of thyroxine, had weak thyroxine activity.

The present communication describes the preparation of diphenyl ethers of types (I) and (II), which possess the same general nuclear structure as thyroxine.



(I.)



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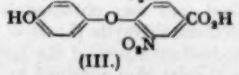


(III.)

The new compounds (I; R = CO_2H or CHO, X = H) were prepared by condensing the appropriately substituted dinitrobenzene with quinol; preparation of (I; R = Me, X = H) has already been described (Borrows, Clayton, Hems, and Long, *J.*, 1949, S 190). Attempts

* Part VII, *J.*, 1950, 2824.

to prepare these compounds by demethylation of their methyl ethers (Borrows *et al.*, *loc. cit.*, p. 190) with hydriodic acid in acetic acid were unsuccessful; demethylation of the methyl ether of (I; R = CO_2H, X = H) by hydrobromic acid in acetic acid took place, but the product contained bromine in the phenolic nucleus, owing to oxidation of the hydrobromic acid by the nitro-groups. The same compound 4-(3-bromo-4-hydroxyphenoxy)-3 : 5-dinitrobenzoic acid (III) was obtained by condensing monobromoquinol with 4-chloro-3 : 5-dinitrobenzoic acid in the usual manner. While it is recognised that this condensation leads to a product of ambiguous constitution, it is thought that, taken in conjunction with the original method of preparation as outlined above, it offers adequate proof of structure. Tars were formed from the methyl ether of (I; R = Me, X = H) by hydriodic or hydrobromic acid in acetic acid or by aluminium chloride in benzene but it was demethylated giving (I; R = Me, X = H) in 53% yield by pyridine hydrochloride at a high temperature.

The substituted propionic acid (I; R = $CH_2 \cdot CH_2 \cdot CO_2H$, X = H) was prepared by condensation of ethyl β-(4-hydroxy-3 : 5-dinitrophenyl)propionate with quinol by the general method described in Part II of this series (Borrows *et al.*, *loc. cit.*, p. 190), followed by acid hydrolysis of the ester, but the preparation of the corresponding unsaturated acid, 4-*p*-hydroxyphenoxy-3 : 5-dinitrocinnamic acid (I; R = $CH{:}CH \cdot CO_2H$, X = H) was much more troublesome. Reaction of the aldehyde (I; R = CHO, X = H) and malonic acid in the presence of bases was anomalous; no pure compound was obtained by use of mixtures of pyridine and piperidine, and replacement of the latter by aniline led only to the formation of 4-anilino-3 : 5-dinitrobenzylidenemalonic acid. The required reaction was, however, successfully accomplished in the presence of pyridine alone or dimethylaniline, thereby avoiding fission of the labile ether linkage.

The dinitrophenyl ethers were then halogenated in the 3′- and the 5′-position by a variety of methods. Bromination proceeded readily with bromine in acetic acid, to give the compounds (I; R = Me, CHO, CO_2H, $CH_2 \cdot CH_2 \cdot CO_2H$, $CH{:}CH \cdot CO_2H$; X = Br). 4-(3 : 5-Dibromo-4-hydroxyphenoxy)-3 : 5-dinitrocinnamic acid (I; R = $CH{:}CH \cdot CO_2H$, X = Br) was also obtained by direct condensation of (I; R = CHO, X = Br) with malonic acid in the presence of pyridine : again no pure product could be isolated if piperidine was also present.

The corresponding chloro-compounds, 4-(3 : 5-dichloro-4-hydroxyphenoxy)-3 : 5-dinitrobenzoic acid and -benzaldehyde (I; R = CO_2H or CHO, X = Cl) were prepared by direct chlorination in acetic acid, but this method failed to give crystalline products from the compounds (I; R = Me, $CH_2 \cdot CH_2 \cdot CO_2H$, $CH{:}CH \cdot CO_2H$, X = H). The failure was attributed to chlorination of the side chain in the presence of excess of halogen, but the two chloro-compounds (I; R = Me or $CH_2 \cdot CH_2 \cdot CO_2H$, X = Cl) were prepared successfully by use of the theoretical amount of chlorine in carbon tetrachloride. The toluene derivative was also obtained by Brown's method (*Ind. Eng. Chem.*, 1944, **36**, 785) using sulphuryl chloride in the presence of aluminium chloride and sulphur chloride. All attempts to prepare the cinnamic acid derivative (I; R = $CH{:}CH \cdot CO_2H$, X = Cl) failed.

Iodination of diphenyl ethers of type (I; X = H) was difficult and no single method was found successful when applied to all the compounds. The use of iodine and aqueous solutions of ammonia or organic primary or secondary bases at room temperature (Clayton and Hems, *J.*, 1950, 840) led to fission of the dinitrodiphenyl ethers. Thus the attempted iodination of (I; R = CO_2H, X = H) in strong aqueous ammonia or in aqueous 20% methylamine solutions led only to 4-amino- or 4-methylamino-3 : 5-dinitrobenzoic acid. The required di-iodo-compounds (I; R = CO_2H and $CH{:}CH \cdot CO_2H$, X = I) were, however, obtained when cold dilute aqueous solutions of ammonia were used. Owing to the insolubility of the compound (I; R = Me, X = H) in dilute aqueous solutions and its cleavage in strong solutions of bases, iodination was achieved in good yield only by adding iodine to the starting material in a mixture of aqueous ammonia with enough added dioxan to secure solution. In this way 4′-hydroxy-3′ : 5′-di-iodo-4-methyl-2 : 6-dinitrodiphenyl ether (I; R = Me, X = I) was obtained without cleavage. The corresponding aldehyde (I; R = CHO, X = I) could not be prepared by this method and was obtained by the action of iodine chloride on 4-*p*-hydroxyphenoxy-3 : 5-dinitrobenzaldehyde (I; R = CHO, X = H) in acetic acid.

Iodo-derivatives of the acidic compounds of this group (I; R = CO_2H and $CH{:}CH \cdot CO_2H$, X = I) were also obtained by the addition of iodine to a solution of the starting material in a saturated solution of sodium hydrogen carbonate.

The preparation of tetrahalogenated diphenyl ethers of type (II; R = various, X = Y = halogen) was carried out by first converting the methyl ethers of compounds of type (I) (Borrows *et al.*, *loc. cit.*, p. 190) into the corresponding halogeno-compounds (II; X = H;

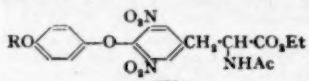
Y = halogen) by catalytic hydrogenation to the diamino-compounds followed by the Sandmeyer reaction. The products after demethylation were then halogenated to give the required compounds (II; X = Y = halogen). In one case (I; R = $CH_2{\cdot}CH_2{\cdot}CO_2H$, X = H) the reactions were carried out with the free phenol.

A method of tetrazotising 2 : 6-diaminodiphenyl ethers was described in Part I (*loc. cit.*), but treating the solutions of tetrazonium salts with iodides under the conditions of the Sandmeyer reaction led to poor yields. Further investigation of this reaction with the improvements already discussed in Part V (*J.*, 1949, 3424) gave increased yields and 2 : 6-di-iodo-4′-methoxy-4-methyldiphenyl ether and the corresponding methyl benzoate have now been thus prepared. Demethylation and, for the ester, hydrolysis were carried out with hydriodic acid and acetic acid to give the hydroxy-derivatives (II; R = Me or CO_2H, X = H, Y = I). The last compound has already been prepared by a different method (Harington and Barger, *Biochem. J.*, 1927, **21**, 169). Similarly, ethyl β-(4-*p*-hydroxyphenoxy-3 : 5-dinitrophenyl)propionate (I; R = $CH_2{\cdot}CH_2{\cdot}CO_2Et$, X = H) was converted into the diamino-compound, thence into ethyl β-(4-*p*-hydroxyphenoxy-3 : 5-di-iodophenyl)propionate, and thus by hydrolysis into (II; R = $CH_2{\cdot}CH_2{\cdot}CO_2H$, Y = I). In the last example tetrazotisation and the Sandmeyer reaction were carried out on the free phenol, and not on the methyl ether, a procedure we have previously found satisfactory (cf. Part V, *loc. cit.*). The three di-iodo-compounds were readily iodinated in aqueous ammonia or ethylamine, to give the tetraiodo-compounds; the benzoic acid derivative has been described by Harington and Barger (*loc. cit.*).

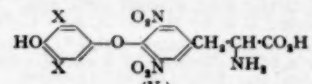
The substituted acrylic acids (II; R = $CH{:}CH{\cdot}CO_2H$, Y = I, X = I or H) have been prepared from thyroxine and 3 : 5-di-iodothyronine by exhaustive methylation: the method will be described later (cf. Wawzonek, Wang, and Lyons, *J. Org. Chem.*, 1950, **15**, 593).

By methods similar to those described above 3 : 5-dichloro-4′-hydroxy-4-methyldiphenyl ether and the corresponding benzoic acid (II; R = Me or CO_2H, X = H, Y = Cl) were prepared. The latter was successfully chlorinated to give (II; R = CO_2H, X = Y = Cl) but all attempts to chlorinate the toluene derivative failed.

The preparation of analogous derivatives of thyronine was then undertaken. Although the methyl ether of *N*-acetyl-3 : 5-dinitro-L-thyronine ethyl ester (IV; R = Me) was readily



(IV.)



(V.)

available (Part V, *loc. cit.*), it did not seem to be suitable for the preparation of 3 : 5-dinitrothyronine itself, in view of the failure to demethylate the methyl ether of (I; R = CO_2H, X = H). However, condensation of *N*-acetyl-3 : 5-dinitro-DL-tyrosine ethyl ester or the L-compound with *p*-benzoyloxyphenol instead of *p*-methoxyphenol (cf. Part V, *loc. cit.*) gave the L- and DL-isomers of *N*-acetyl-4-*p*-benzoyloxyphenoxy-3 : 5-dinitrophenylalanine ethyl ester (IV; R = Bz). Acid hydrolysis readily yielded the 3 : 5-dinitro-L- and -DL-thyronine (V; X = H) which were nitrated to give 3 : 5 : 3′ : 5′-tetranitro-L- and -DL-thyronine (V; X = NO_2) respectively. The free amino-acids did not crystallise well and were characterised as their *N*-acetyl derivatives. 3 : 5-Dinitro-DL-thyronine with iodine in sodium hydrogen carbonate gave a product that appeared from its analysis to consist essentially of the di-iodo-compound (V; X = I), but it could not be obtained pure, and in this instance acetylation did not lead to a more tractable substance.

Experimental.

4-p-*Hydroxyphenoxy*-3 : 5-*dinitrobenzaldehyde*.—A mixture of quinol (2·7 g.), sodium carbonate (0·26 g.), 4-chloro-3 : 5-dinitrobenzaldehyde (1·15 g.), sodium dithionite (hydrosulphite) (0·05 g.), and water (10 ml.) was heated under reflux for 1 hour. The reaction mixture was diluted with water (250 ml.), then filtered, and the solid was extracted with hot acetic acid. After filtration a little water was added, whereupon the *aldehyde* separated as yellow needles, m. p. 157° (0·5 g.) (Found: C, 51·5; H, 2·1; N, 9·5. $C_{13}H_8O_7N_2$ requires C, 51·3; H, 2·6; N, 9·2%).

4-p-*Hydroxyphenoxy*-3 : 5-*dinitrobenzoic Acid*.—Quinol (2·25 g.), potassium carbonate (0·56 g.), 4-chloro-3 : 5-dinitrobenzoic acid (1 g.), and water (5 ml.) were heated under reflux for an hour. The mixture was poured into water and acidified with 2N-hydrochloric acid, yellow crystals being deposited. The *acid* recrystallised from aqueous ethanol as bright yellow prisms, m. p. 224° (decomp.) (0·9 g., 69%) (Found: C, 48·2; H, 2·7; N, 8·9. $C_{13}H_8O_8N_2$ requires C, 48·7; H, 2·5; N, 8·7%).

4′-*Hydroxy*-4-*methyl*-2 : 6-*dinitrodiphenyl Ether*.—A solution of 4′-methoxy-2 : 6-dinitro-4-methyldiphenyl ether (1 g.) in benzene (50 ml.) and pyridine (1 ml.) was saturated with dry hydrogen chloride and then heated to 220—225° for 1 hour, the solvent being allowed to evaporate. The solid was cooled

and extracted thoroughly with hot benzene, and the extracts were washed with dilute hydrochloric acid, sodium carbonate solution, and water and evaporated. The residual gum crystallised from aqueous acetic acid as yellow needles, m. p. 160—161° (0·5 g., 53%) alone or mixed with an authentic specimen (Borrows *et al.*, *J.*, 1949, S 190).

4-(3-*Bromo*-4-*hydroxyphenoxy*)-3 : 5-*dinitrobenzoic Acid*.—(*a*) **4-*p*-Methoxyphenoxy-3 : 5-dinitrobenzoic acid** (*loc. cit.*) (2 g.) in acetic acid (14 ml.) and hydrobromic acid (*d* 1·5; 10 ml.) was heated under reflux for 15 hours. The brown solid which separated on pouring of the mixture into water was recrystallised from aqueous ethanol; the *acid* separated as brown crystals, m. p. 227—230° (decomp.) (Found: C, 39·6; H, 1·9; N, 7·0; Br, 19·6. $C_{13}H_7O_8N_2Br$ requires C, 39·1; H, 1·8; N, 7·0; Br, 20·0%).

(*b*) A mixture of potassium carbonate (0·28 g.), bromoquinol (1 g.), 4-chloro-3 : 5-dinitrobenzoic acid (0·5 g.), and water (5 ml.) was heated under reflux for 1 hour, then poured into water, and acidified with 2N-hydrochloric acid, whereupon a sticky solid was deposited. Crystallisation from aqueous ethanol gave a yellow solid (0·1 g.) which had m. p. 228° (decomp.), not depressed on admixture with a sample of the acid prepared by demethylation.

Ethyl **β-(4-p-*Hydroxyphenoxy*-3 : 5-*dinitrophenyl*)*propionate*.**—Ethyl β-(4-hydroxy-3 : 5-dinitrophenyl)-propionate (5 g.) and toluene-*p*-sulphonyl chloride (3·35 g.) were heated on the steam-bath with pyridine (100 ml.) for 10 minutes. Quinol (8 g.) was added and the solution heated under reflux for 1 hour; the pyridine was then removed under reduced pressure and ethanol (80 ml.) added to the residual gum. The solution was filtered, water added, and the mixture extracted with chloroform. The extracts were washed with water and evaporated to small bulk; addition of light petroleum caused the separation of yellow prisms of the product. The *ester*, recrystallised from aqueous acetic acid, had m. p. 79—82° (4·35 g., 65%) (Found: C, 52·3; H, 4·6; N, 6·4. $C_{17}H_{16}O_8N_2,CH_3{\cdot}CO_2H$ requires C, 52·3; H, 4·6; N, 6·4%). The *acid* was obtained by heating the ester (10 g.) under reflux with acetic acid (50 ml.) and hydrochloric acid (50 ml.) for 30 minutes. The product separated from aqueous ethanol as yellow prisms, m. p. 184—186° (7·6 g., 82%) (Found: C, 52·0; H, 3·4; N, 8·1. $C_{15}H_{13}O_8N_2$ requires C, 51·7; H, 3·5; N, 8·0%).

4-*Anilino*-3 : 5-*dinitrobenzylidenemalonic Acid*.—**4-*p*-Hydroxyphenoxy-3 : 5-dinitrobenzaldehyde** (1 g.), malonic acid (2 g.), and aniline (0·3 ml.) in pyridine (25 ml.) were warmed on a steam-bath for 30 minutes and heated under reflux for 15 minutes. The mixture was poured into excess of dilute hydrochloric acid; the orange crystals which separated recrystallised from aqueous ethanol and then had m. p. 256—258° (Found: C, 51·6; H, 3·1; N, 11·2. $C_{16}H_{11}O_8N_3$ requires C, 51·5; H, 3·0; N, 11·3%).

4-p-*Hydroxyphenoxy*-3 : 5-*dinitrocinnamic Acid*.—**4-*p*-Hydroxyphenoxy-3 : 5-dinitrobenzaldehyde** (2 g.), malonic acid (3 g.), and dry pyridine (25 ml.) were heated on a steam-bath for 1 hour and then under reflux for 30 minutes. The solution was poured into excess of dilute hydrochloric acid and the *acid* was recrystallised from aqueous acetic acid. It formed yellow prisms, m. p. 225° (decomp.) (1·8 g., 80%) (Found: C, 51·6; H, 3·0; N, 7·8. $C_{15}H_{10}O_8N_2$ requires C, 52·0; H, 2·9; N, 8·1%).

3′ : 5′-*Dibromo*-4′-*hydroxy*-4-*methyl*-2 : 6-*dinitrodiphenyl Ether*.—Bromine in acetic acid (10% w/v; 42 ml.) was added dropwise to a stirred solution of 4′-hydroxy-4-methyl-2 : 6-dinitrodiphenyl ether (2 g.) in acetic acid (50 ml.) during 15 minutes. After 1 hour, water was added and the yellow crystalline *ether* which was deposited was recrystallised from ethanol as glistening plates, m. p. 194—195° (2·1 g., 69%) (Found: C, 35·0; H, 2·0; N, 6·0; Br, 35·4. $C_{13}H_8O_6N_2Br_2$ requires C, 34·8; H, 1·8; N, 6·2; Br, 35·7%).

By similar methods the following compounds were prepared.

4-(3 : 5-*Dibromo*-4-*hydroxyphenoxy*)-3 : 5-*dinitrobenzoic acid*, yellow prisms (from aqueous ethanol), m. p. 259° (decomp.) (Found: N, 5·7; Br, 32·8. $C_{13}H_6O_8N_2Br_2$ requires N, 5·9; Br, 33·5%).

4-(3 : 5-*Dibromo*-4-*hydroxyphenoxy*)-3 : 5-*dinitrobenzaldehyde*, yellow prisms (from aqueous acetic acid), m. p. 186—187° (decomp.) (Found: C, 33·2; H, 1·2; N, 6·1; Br, 34·0. $C_{13}H_6O_7N_2Br_2$ requires C, 33·8; H, 1·3; N, 6·1; Br, 34·6%).

β-[4-(3 : 5-*Dibromo*-4-*hydroxyphenoxy*)-3 : 5-*dinitrophenyl*]*propionic acid*, yellow prisms (from aqueous ethanol), m. p. 173—175° (Found: C, 36·1; H, 2·3; N, 5·5; Br, 30·5. $C_{15}H_{10}O_8N_2Br_2$ requires C, 35·6; H, 2·0; N, 5·5; Br, 31·6%).

4-(3 : 5-*Dibromo*-4-*hydroxyphenoxy*)-3 : 5-*dinitrocinnamic acid*, yellow prisms (from aqueous acetic acid), m. p. 260° (decomp.) (Found: C, 36·3; H, 1·5; N, 5·7; Br, 31·2. $C_{15}H_8O_8N_2Br_2$ requires C, 35·7; H, 1·6; N, 5·6; Br, 31·7%).

4-(3 : 5-*Dibromo*-4-*hydroxyphenoxy*)-3 : 5-*dinitrocinnamic Acid*.—**4-(3 : 5-Dibromo-4-hydroxyphenoxy)-3 : 5-dinitrobenzaldehyde** (2 g.) and malonic acid (5 g.) in dry pyridine (40 ml.) were heated on a steam-bath for 1 hour and then under reflux for 10 minutes. The pyridine was removed under reduced pressure and the residue triturated with a little acetic acid, whereupon a yellow solid separated having m. p. and mixed m. p. 260° (decomp.).

3′ : 5′-*Dichloro*-4′-*hydroxy*-4-*methyl*-2 : 6-*dinitrodiphenyl Ether*.—(*a*) **4′-Hydroxy-4-methyl-2 : 6-dinitrodiphenyl ether** (1 g.) was dissolved in acetic acid (50 ml.); sulphuryl chloride (2 ml.), aluminium chloride (2 g.), and sulphur chloride (0·05 ml.) were added and the mixture was stirred for 15 hours. Addition of water caused the precipitation of a yellow solid *ether* which separated from aqueous acetic acid as yellow prisms, m. p. 151° (decomp.) (Found: N, 7·8; Cl, 19·7. $C_{13}H_8O_6N_2Cl_2$ requires N, 7·8; Cl, 19·8%).

(*b*) To **4′-hydroxy-4-methyl-2 : 6-dinitrodiphenyl ether** (1 g.) in acetic acid (50 ml.) the calculated amount of chlorine solution in carbon tetrachloride (13·3% w/v; 3·65 ml.) was added. After the reactants had been kept in the dark for 1 hour, water was added, precipitating a solid which separated from aqueous acetic acid as yellow prisms (0·66 g., 53%), m. p. 149—152° (decomp.), alone or mixed with a sample prepared as in (*a*) above.

4-(3 : 5-*Dichloro*-4-*hydroxyphenoxy*)-3 : 5-*dinitrobenzaldehyde*.—Dry chlorine was passed into a solution of 4-*p*-hydroxyphenoxy-3 : 5-dinitrobenzaldehyde (1 g.) in acetic acid (50 ml.) for 1½ hours. Addition of water precipitated the *aldehyde* which recrystallised from benzene as yellow prisms, m. p. 207—208° (Found : C, 41·5; H, 1·6; N, 7·8; Cl, 18·6. $C_{13}H_4O_7N_2Cl_2$ requires C, 41·8; H, 1·6; N, 7·5; Cl, 19·0%).

4-(3 : 5-*Dichloro*-4-*hydroxyphenoxy*)-3 : 5-*dinitrobenzoic Acid*.—Dry chlorine was passed into a solution of 4-*p*-hydroxyphenoxy-3 : 5-dinitrobenzoic acid (1 g.) in acetic acid (50 ml.) for 1½ hours. The solution was concentrated to small bulk and when this was kept a solid *acid* separated which recrystallised from aqueous acetic acid as glistening yellow plates, m. p. 250° (decomp.) (0·61 g., 50%) (Found : C, 40·4; H, 1·6; N, 7·4; Cl, 17·8. $C_{13}H_4O_8N_2Cl_2$ requires C, 40·2; H, 1·5; N, 7·2; Cl, 18·2%).

β-[4-(3 : 5-*Dichloro*-4-*hydroxyphenoxy*)3 : 5-*dinitrophenyl*]*propionic Acid*.—Chlorine in carbon tetrachloride (8·2% w/v; 25 ml.) was added to a solution of β-4-(*p*-hydroxyphenoxy)-3 : 5-dinitrophenylpropionic acid (5 g.) in acetic acid (250 ml.), and the solution kept in the dark for 1 hour. Addition of water precipitated the *acid* which recrystallised from aqueous ethanol as yellow prisms, m. p. 177—179° (3·2 g., 53%) (Found : C, 43·2; H, 2·5; N, 6·8; Cl, 16·4. $C_{15}H_{10}O_8N_2Cl_2$ requires C, 43·2; H, 2·4; N, 6·7; Cl, 17·0%).

4-*Amino*-3 : 5-*dinitrobenzoic Acid*.—4-*p*-Hydroxyphenoxy-3 : 5-dinitrobenzoic acid (1 g.) was dissolved in ammonia solution (*d* 0·88; 20 ml.) and the solution set aside for 10 minutes. Acidification with 2N-hydrochloric acid caused the separation of a brown solid which recrystallised from ethyl acetate–light petroleum as yellow crystals, m. p. 269—271° (Found : N, 18·4. Calc. for $C_7H_5O_6N_3$: N, 18·5%).

4-*Methylamino*-3 : 5-*dinitrobenzoic acid*.—4-*p*-Hydroxyphenoxy-3 : 5-dinitrobenzoic acid (1 g.) was dissolved in methylamine solution (20% w/v; 20 ml.), set aside for 10 minutes, and then acidified with 2N-hydrochloric acid. The precipitated amino-acid crystallised from aqueous acetone as yellow prisms, m. p. 223—224° (Found : C, 40·2; H, 2·9; N, 17·6. Calc. for $C_8H_7O_6N_3$: C, 39·9; H, 2·9; N, 17·4%).

4-(4-*Hydroxy*-3 : 5-*di-iodophenoxy*)-3 : 5-*dinitrobenzoic Acid*.—(*a*) Iodine solution (1·9N.) in an excess of sodium iodide (13·2 ml.) was added dropwise to a stirred solution of 4-*p*-hydroxyphenoxy-3 : 5-dinitrobenzoic acid (2 g.) in a saturated solution of sodium hydrogen carbonate (100 ml.). After 30 minutes the mixture was acidified with dilute hydrochloric acid; the resultant yellow precipitate of *iodo-acid* separated from aqueous ethanol as bright yellow crystals, m. p. 241° (decomp.) (Found : C, 27·5; H, 1·3; N, 5·1; I, 44·3. $C_{13}H_6O_8N_2I_2$ requires C, 27·3; H, 1·1; N, 4·9; I, 44·4%).

(*b*) 4-*p*-Hydroxyphenoxy-3 : 5-dinitrobenzoic acid (1 g.) was dissolved in ammonia solution [50 ml.; 25% (v/v) of ammonia (*d* 0·88) in water] at −5°. A solution of iodine (1·9N.) in an excess of sodium iodide solution (6·6 ml.) was added dropwise, the temperature being kept between −5° and 0°. The solution was poured into a large excess of water and acidified with 2N-hydrochloric acid, whereupon a sticky yellow solid separated. Recrystallisation from aqueous ethanol yielded yellow prisms, m. p. 246° (decomp.) (1·0 g., 55%).

4′-*Hydroxy*-3′ : 5′-*di-iodo*-4-*methyl*-2 : 6-*dinitrodiphenyl Ether*.—To a stirred solution of 4′-hydroxy-4-methyl-2 : 6-dinitrodiphenyl ether (9·3 g.) in dioxan (110 ml.) and ammonia (*d* 0·88; 93 ml.), iodine solution in excess of sodium iodide solution (2N.; 59 ml.) was added. The mixture was poured into dilute hydrochloric acid, and the precipitated *ether* was recrystallised from acetone, forming yellow prisms, m. p. 206—207° (decomp.) (11 g., 69%) (Found : C, 29·5; H, 1·4; N, 5·3; I, 45·6. $C_{19}H_9O_4N_2I_2$ requires C, 28·8; H, 1·5; N, 5·2; I, 46·8%).

4-(4-*Hydroxy*-3 : 5-*di-iodophenoxy*)-3 : 5-*dinitrobenzaldehyde*.—Iodine monochloride in acetic acid (15% w/v; 7·2 ml.) was added to a solution of 4-*p*-hydroxyphenoxy-3 : 5-dinitrobenzaldehyde (1 g.) in acetic acid (50 ml.), and the mixture stirred for 15 hours. Addition of excess of water precipitated a brown solid *aldehyde* which recrystallised from aqueous acetic acid as fine yellow needles, m. p. 180° (decomp.) (0·6 g., 30%) (Found : N, 5·1; I, 45·3. $C_{13}H_6O_7N_2I_2$ requires N, 5·0; I, 45·7%).

4-(4-*Hydroxy*-3 : 5-*di-iodophenoxy*)-3 : 5-*dinitrocinnamic Acid*.—Iodine in excess of sodium iodide solution (1·9N.; 32 ml.) was added dropwise to a stirred solution of 4-*p*-hydroxyphenoxy-3 : 5-dinitrocinnamic acid (7·8 g.) in saturated sodium hydrogen carbonate solution (100 ml.). After 30 minutes the mixture was acidified with dilute hydrochloric acid and the resultant *acid* crystallised from aqueous methyl cyanide as yellow needles, m. p. 226° (decomp.) (6·0 g., 45%) (Found : C, 30·2; H, 1·5; N, 4·9; I, 43·0. $C_{15}H_8O_8N_2I_2$ requires C, 30·1; H, 1·4; N, 4·7; I, 42·4%).

β-[4-(4-*Hydroxy*-3 : 5-*di-iodophenoxy*)-3 : 5-*dinitrophenyl*]*propionic Acid*.—Iodine in excess of sodium iodide solution (1·9N.; 12·1 ml.) was added rapidly to a stirred solution of β-(4-*p*-hydroxyphenoxy-3 : 5-dinitrophenyl)propionic acid (2 g.) in aqueous ammonia (*d* 0·88; 20 ml.) at 0°. After 5 minutes the solution was acidified with hydrochloric acid and the resultant precipitate was recrystallised from benzene, forming yellow needles, m. p. 189—191° (0·7 g., 20%) (Found : C, 33·8; H, 2·2; N, 4·2; I, 40·0. $C_{15}H_{10}O_8N_2I_2,0{\cdot}5C_6H_6$ requires C, 33·8; H, 2·0; N, 4·4; I, 39·7%).

2 : 6-*Di-iodo*-4′-*methoxy*-4-*methyldiphenyl Ether* (cf. Part I, *loc. cit.*).—2 : 6-Diamino-4′-methoxy-4-methyldiphenyl ether dihydrochloride (5 g.) was dissolved in acetic acid (100 ml.), and the solution added with cooling to sulphuric acid (130 ml.). This solution was added during 30 minutes to a stirred solution of sodium nitrite (2·5 g.) in sulphuric acid (50 ml.) and acetic acid (50 ml.), the temperature being kept at −5°; stirring was continued for 1½ hours at −2°. The tetrazonium salt solution was added dropwise to sodium iodide (20 g.) and iodine (20 g.) in water (700 ml.) and chloroform (100 ml.) with vigorous stirring. After being kept overnight the organic phase was separated, washed with sodium hydrogen sulphite solution and water, and then evaporated. The residue was taken up in benzene, and the solution chromatographed on a column of alumina. Evaporation of the eluate left a gum which crystallised from ethanol as prisms (3·89 g., 52%), m. p. 110—112°, undepressed on admixture with an authentic specimen.

4'-Hydroxy-2 : 6-di-iodo-4-methyldiphenyl Ether.—2 : 6-Di-iodo-4'-methoxy-4-methyldiphenyl ether (1 g.) in acetic acid (12 ml.) and hydriodic acid (*d* 1·7; 5 ml.) was heated under reflux for 1 hour. Addition of water precipitated a solid *phenol* which separated from aqueous acetic acid as prisms, m. p. 150—151° (0·8 g., 83%) (Found: C, 34·5; H, 2·4; I, 56·2. $C_{13}H_{10}O_3I_2$ requires C, 34·5; H, 2·2; I, 56·1%).

4-p-*Hydroxyphenoxy-3 : 5-di-iodobenzoic Acid.*—Methyl 3 : 5-di-iodo-4-*p*-methoxyphenoxybenzoate (10 g.) in acetic acid (70 ml.) and hydriodic acid (*d* 1·7; 40 ml.) was heated under reflux for 1 hour. On cooling, the product separated as minute crystals, m. p. 260° (6·34 g., 67%) (Found: I, 52·8. Calc. for $C_{13}H_8O_4I_2$: I, 52·7%) (Harington and Barger, *loc. cit.*, give m. p. 250—254°).

Ethyl β-(4-p-*Hydroxyphenoxy-3 : 5-di-iodophenyl)propionate.*—Ethyl β-(4-*p*-hydroxyphenoxy-3 : 5-dinitrophenyl)propionate (7·9 g.) in acetic acid (200 ml.) was hydrogenated at room temperature and pressure over palladised charcoal (10%; 2 g.). The diamine solution was filtered and added dropwise during 30 minutes to a stirred solution of sodium nitrite (6·8 g.) in acetic acid (100 ml.) and sulphuric acid (100 ml.) at 0°. After a further hour's stirring the tetrazonium salt solution was added dropwise to a solution of iodine (20 g.) and sodium iodide (30 g.) in water (400 ml.) with vigorous stirring, while the temperature was kept below 40°. Next morning the mixture was extracted with chloroform, and the extract shaken with sodium metabisulphite solution and evaporated to dryness. The residue was dissolved in benzene (100 ml.) and chromatographed on a column of alumina. Elution was achieved by using a solution of methanol (10% w/v) in benzene, and the eluate was concentrated to 20 ml. Addition of light petroleum caused the separation of the desired *ester* as pale brown prisms, m. p. 153—156° (5·6 g., 50%) (Found: C, 38·3; H, 3·1; I, 46·9. $C_{17}H_{16}O_4I_2$ requires C, 37·9; H, 3·0; I, 47·2%).

β-(4-p-*Hydroxyphenoxy-3 : 5-di-iodophenyl)propionic Acid.*—The foregoing ester (4·6 g.), dissolved in acetic acid (50 ml.) and hydrochloric acid (40 ml.), was heated under reflux for 1 hour. On cooling, the phenolic *acid* separated as buff-coloured crystals, m. p. 250° (4·1 g., 94%) (Found: I, 50·2. $C_{15}H_{12}O_4I_2$ requires I, 49·8%).

4'-Hydroxy-2 : 6 : 3' : 5'-tetraiodo-4-methyldiphenyl Ether.—Iodine in excess of sodium iodide solution (1·9N.; 4·5 ml.) was added dropwise to a stirred suspension of 4'-hydroxy-2 : 6-di-iodo-4-methyldiphenyl ether (1 g.) in aqueous ethylamine (33%; 10 ml.). After 2 hours the mixture was acidified with hydrochloric acid, and a little sodium metabisulphite added to remove free iodine. Recrystallisation of the resultant solid from aqueous ethanol yielded the *ether* as prisms m. p. 198—200° (0·3 g., 20%) (Found: I, 72·1. $C_{13}H_8O_2I_4$ requires I, 72·1%).

4-(4-*Hydroxy-3 : 5-di-iodophenoxy)-3 : 5-di-iodobenzoic Acid.*—Iodine in excess of sodium iodide solution (1·9N.; 41 ml.) was added dropwise to a stirred solution of 4-*p*-hydroxyphenoxy-3 : 5-di-iodobenzoic acid (10 g.) in ammonia (*d* 0·88; 500 ml.) during 30 minutes. After 1 hour the solution was acidified with hydrochloric acid, and the precipitate recrystallised from aqueous acetone (charcoal) from which it separated as white crystals, m. p. 267—269° (decomp.) (12·8 g., 84%) (Found: I, 69·8. Calc. for $C_{13}H_6O_4I_4$: I, 69·2%) (Harington and Barger, *loc. cit.*, give m. p. 255°).

β-[4-(4-*Hydroxy-3 : 5-di-iodophenoxy)-3 : 5-di-iodophenyl]propionic Acid.*—To a stirred solution of β-(4-*p*-hydroxyphenoxy-3 : 5-di-iodophenyl)propionic acid (5 g.) in aqueous ethanol (33%; 100 ml.), a solution of iodine in excess of aqueous sodium iodide (1·9N.; 21 ml.) was added dropwise during 30 minutes. After 1 hour hydrochloric acid was added; the precipitated solid *acid* recrystallised from aqueous acetone as minute white crystals, m. p. 218—222° (7 g., 94%) (Found: C, 24·1; H, 1·6; I, 67·3. $C_{15}H_{10}O_4I_4$ requires C, 23·7; H, 1·3; I, 66·6%).

2 : 6-*Dichloro-4'-hydroxy-4-methyldiphenyl Ether.*—2 : 6-Diamino-4'-methoxy-4-methyldiphenyl ether dihydrochloride (10 g.) in acetic acid (100 ml.) was added dropwise during 30 minutes to a stirred solution of sodium nitrite (4·1 g.) in sulphuric acid (72 ml.) at 0°. After 2 hours' stirring the solution was added at 0° to a solution of cuprous chloride, prepared by heating under reflux cupric sulphate (10·8 g.), sodium chloride (5·5 g.), copper powder (6 g.), and concentrated hydrochloric acid (150 ml.). Next morning the product was extracted with benzene, and the benzene extracts after drying were passed down a column of alumina, the colourless eluate being collected. Evaporation yielded a gum and on treatment with ethanol crystals of 2 : 6-dichloro-4'-methoxy-4-methyldiphenyl ether, m. p. 76—78°, separated. A satisfactory microanalysis was not obtained and the ether was demethylated by heating it under reflux for 1 hour with acetic acid (60 ml.) and hydriodic acid (*d* 1·7; 25 ml.), to give 2 : 6-*dichloro-4'-hydroxy-4-methyldiphenyl ether*, m. p. 110—111° (4·5 g.) (Found: Cl, 26·6. $C_{13}H_{10}O_2Cl_2$ requires Cl, 26·4%).

3 : 5-*Dichloro-4-p-hydroxyphenoxybenzoic Acid.*—This compound was prepared from methyl 3 : 5-diamino-4-*p*-methoxyphenoxybenzoate by the procedure described above for the toluene derivative. The *acid* formed prisms, m. p. 223—225°, from acetic acid (Found: C, 52·3; H, 2·9; Cl, 23·1. $C_{13}H_8O_4Cl_2$ requires C, 52·2; H, 2·7; Cl, 23·7%).

3 : 5-*Dichloro-4-(3 : 5-dichloro-4-hydroxyphenoxy)benzoic Acid.*—3 : 5-Dichloro-4-*p*-hydroxyphenoxybenzoic acid (1 g.) was dissolved in acetic acid (10 ml.) and chlorine passed in for 3 hours. The acetic acid was evaporated under reduced pressure and the residual yellow oily *tetrachloro-acid* was induced to crystallize from aqueous methyl cyanide. It formed buff-coloured prisms, m. p. 216—218° (0·2 g., 16%) (Found: Cl, 38·6. $C_{13}H_4O_4Cl_4$ requires Cl, 38·5%).

3 : 5-*Dinitro-*DL-*tyrosine.*—DL-Tyrosine (50 g.) was added to sulphuric acid (200 ml.) with stirring, the temperature being kept below 10°. Nitric acid (*d* 1·42; 45 ml.) was added dropwise during 45 minutes, the temperature being kept below 15°. After a further 30 minutes the mixture was poured on ice (1 kg.), and sodium hydroxide solution (40%; 750 ml.) was added until the pH was 4—5. The red *monosodium* salt was filtered off hot (69 g., 75%) (Found: N, 13·0; Na, 7·0. $C_9H_6O_7N_3Na,2H_2O$ requires N, 12·8; Na, 7·0%). The sodium salt was suspended in methanol (500 ml.), and methanolic hydrogen chloride added until the hydrochloride dissolved on warming. The solution was evaporated to dryness after clarification (charcoal), and taken up in water, whereupon the free *amino-acid* separated (Found: C, 37·2; H, 4·1; N, 14·2. $C_9H_9O_7N_3,H_2O$ requires C, 37·4; H, 3·9; N, 14·5%).

N-Acetyl-3 : 5-dinitro-DL-tyrosine.—3 : 5-Dinitro-DL-tyrosine (10 g.) was dissolved in 2N-sodium hydroxide (80 ml.) and stirred whilst acetic anhydride (6 ml.) was added dropwise during 30 minutes, the temperature being below 20°. After 1 hour's stirring the solution was warmed to 40° to decompose excess of anhydride, and acidified, bright yellow prisms, m. p. 209° (8·7 g., 76%), being precipitated (Found : C, 42·3; H, 3·5; N, 13·4. $C_{11}H_{11}O_8N_3$ requires C, 42·2; H, 3·6; N, 13·4%).

N-Acetyl-3 : 5-dinitro-DL-tyrosine Ethyl Ester.—(*a*) The acid (25 g.) was dissolved in chloroform (500 ml.)–ethyl alcohol (60 ml.), and toluene-*p*-sulphonic acid (2 g.) added. The solution was heated under reflux for 8 hours under an azeotropic water-removal head, 30 ml. more ethanol being added after 3 hours. The cooled solution was extracted twice with 2N-sodium carbonate, and the extract acidified with hydrochloric acid. The thick brown oil which was precipitated, solidified upon scratching. The resultant *ester* separated from ethanol as yellow crystals, m. p. 129—130° (Found : C, 45·9; H, 4·6; N, 11·6. $C_{13}H_{15}O_8N_3,\frac{1}{2}EtOH$ requires C, 46·2; H, 5·0; N, 11·5%).

(*b*) This compound was also prepared by racemisation of the corresponding L-compound. *N*-Acetyl-3 : 5-dinitro-L-tyrosine ethyl ester (6·82 g.) was added to a solution of sodium (1 g.) in ethanol (100 ml.). After 4 hours' mechanical shaking chloroform (120 ml.) was added and to the vigorously stirred mixture N-hydrochloric acid (44 ml.) was added with cooling. The chloroform layer was separated, washed, and evaporated, to leave a gum which separated from ethanol as yellow needles, m. p. 129° (5·6 g., 81%). The material was optically inactive.

N-Acetyl-4-p-benzoyloxyphenoxy-3 : 5-dinitro-DL-phenylalanine Ethyl Ester.—The acetyl ester (20 g.), toluene-*p*-sulphonyl chlorde (12·4 g.), and pyridine (100 ml.) were heated on the steam-bath for 30 minutes. *p*-Benzoyloxyphenol (25 g.) was added and the solution heated under reflux for 1 hour. To the cold mixture chloroform (150 ml.) was added and the mixture washed with 2N-hydrochloric acid and 2N-sodium carbonate. After evaporation of the chloroform layer the resultant solid *ester* was crystallised from ethanol, forming buff-coloured prisms, m. p. 173—175° (23 g., 72%) (Found : C, 58·2; H, 4·2; N, 7·8. $C_{24}H_{22}O_{10}N_2$ requires C, 58·1; H, 4·3; N, 7·8%).

The corresponding *L-compound* was prepared in the same way. It formed feathery pale yellow crystals, m. p. 159—160° (Found : C, 58·0; H, 4·35; N, 7·6. $C_{24}H_{22}O_{10}N_2$ requires C, 58·1; H, 4·3; N, 7·8%), and had $[\alpha]_D^{28}$ −12° (*c*, 1·2% in dioxan).

3 : 5-Dinitro-DL-thyronine.—*N*-Acetyl-4-*p*-benzoyloxyphenoxy-3 : 5-dinitro-DL-phenylalanine ethyl ester (5 g.) was heated in acetic acid (20 ml.) and hydrobromic acid (20 ml.; *d* 1·49) under reflux for 1 hour. After evaporation under reduced pressure the *hydroxy*-compound was crystallised from water from which it separated as yellow prisms, m. p. 230° (decomp.) (2·9 g., 85%) (Found : C, 47·6; H, 4·4; N, 11·3. $C_{15}H_{13}O_8N_3,H_2O$ requires C, 47·3; H, 4·0; N, 11·0%).

3 : 5-Dinitro-L-thyronine, prepared similarly, formed yellow-brown prisms, m. p. 215° (decomp.) (Found : C, 49·3; H, 3·6; N, 11·6. $C_{15}H_{13}O_8N_3$ requires C, 49·6; H, 3·6; N, 11·6%), and had $[\alpha]_D^{29}$ +20° (*c*, 0·7% in N-hydrochloric acid).

3 : 5 : 3′ : 5′-Tetranitro-DL-thyronine.—3 : 5-Dinitro-DL-thyronine (1 g.) was added in portions to sulphuric acid (60 ml.), stirred at 10°. The solution was cooled to 0° and nitric acid (0·37 ml.; *d* 1·4) was added portionwise. After 30 minutes' stirring at 0°, the solution was poured on ice and the resultant yellow solid filtered off and dissolved in boiling water (20 ml.); on cooling, orange-yellow needles separated, having m. p. 222° (decomp.). Recrystallisation proved troublesome and a satisfactory analysis was not obtained (Found : C, 35·6; H, 2·3; N, 13·6. $C_{15}H_{11}O_{12}N_4,3H_2O$ requires C, 35·5; H, 3·4; N, 13·8%).

N-Acetyl-3 : 5 : 3′ : 5′-tetranitro-DL-thyronine.—The free amino-acid (2 g.) was suspended in a saturated solution of sodium hydrogen carbonate (50 ml.), and the mixture stirred during the addition of acetic anhydride (2·5 ml.). The solid gradually dissolved and the solution was acidified with 2N-hydrochloric acid. The *acetyl* derivative which was precipitated crystallised from ethanol as yellow prisms, m. p. 230—231° (Found : C, 41·0; H, 2·8; N, 13·8. $C_{17}H_{13}O_{13}N_4$ requires C, 41·2; H, 2·6; N, 14·15%).

3 : 5 : 3′ : 5′-Tetranitro-L-thyronine.—This *compound* was prepared by the method described above for the corresponding DL-compound. It formed yellow orange prisms, m. p. 225° (decomp.) (Found : C, 39·7; H, 3·0; N, 14·8. $C_{15}H_{11}O_{12}N_4$ requires C, 39·7; H, 2·5; N, 15·45%). No rotation measurement was carried out as the material was insufficiently soluble in hydrochloric acid and formed a dark red solution in alkali.

N-Acetyl-3 : 5 : 3′ : 5′-tetranitro-L-thyronine.—Acetylation was carried out as described above. The *product* formed yellow prisms, m. p. 237° (decomp.), $[\alpha]_D^{28}$ −22° (*c*, 0·2% in dioxan) (Found : C, 41·3; H, 2·9; N, 13·7%).

3′ : 5′-Di-iodo-3 : 5-dinitro-DL-thyronine.—To 3 : 5-dinitro-DL-thyronine (1 g.) in a saturated solution of sodium hydrogen carbonate (100 ml.) a solution of iodine in excess of sodium iodide solution (1·9N.; 58 ml.) was added. After 20 minutes the solution was brought to pH 5 by addition of hydrochloric acid; a yellow precipitate slowly formed. The material was recrystallised by dissolving it in ethanol–hydrochloric acid, removing the ethanol by evaporation, and adjusting the pH to 5 by sodium acetate solution. The solid *iodo*-compound obtained after a number of such crystallisations had m. p. 206—207° (decomp.), but did not give a satisfactory analysis (Found : C, 28·3; H, 2·2; N, 5·6; I, 40·6. $C_{15}H_{11}O_8N_3I_2$ requires C, 29·3; H, 1·8; N, 6·8; I, 41·3%).

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545. *Lanosterol. Part XII.* The Constitution of Some Oxidation Products from Lanostadienol and Lanostenol.*

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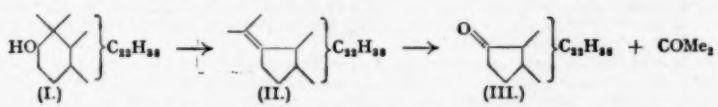
A reinvestigation of the acidic oxidation products reported by Marker, Wittle, and Mixon (*J. Amer. Chem. Soc.*, 1937, **59**, 1368) and by Wieland and Joost (*Annalen*, 1941, **548**, 103) has shown that they may be formulated as $C_{27}H_{40}O_5$ and $C_{30}H_{50}O_4$, respectively. Attention is drawn to conflicting reports of acidic degradation products from euphadienyl acetate together with a suggested reinterpretation.

In recent years considerable progress has been made in the elucidation of the structure of the diolefinic alcohol, lanostadienol, owing largely to the work of Ruzicka and Jeger and their co-workers in Switzerland and of Dorée and McGhie and their co-workers in this country. As a result of these investigations the environment of the secondary alcohol grouping has been established, and the reactive ethylenic linkage shown to be present in an exocyclic *iso*propylidene group. These conclusions have been based on the following experimental facts.

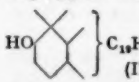
Dehydration of lanostenol ($C_{30}H_{52}O$) with phosphorus pentachloride gave an unsaturated hydrocarbon, *iso*lanostadiene $C_{30}H_{50}$ (Dorée, McGhie, and Kurzer, *J.*, 1947, 1467), which could not be hydrogenated to lanostene; it was postulated that in its formation a retropinacoline change had taken place. Oxidation of this hydrocarbon with osmium tetroxide followed by fission of the two isomeric glycols, so obtained, with lead tetra-acetate gave the A-norketone,† $C_{27}H_{44}O$, together with acetone, which was isolated and identified as the 2 : 4-dinitrophenylhydrazone (Dorée, McGhie, and Kurzer, *Nature*, 1949, **163**, 140; *J.*, 1949, S 167; see also Ruzicka, Montavon, and Jeger, *Helv. Chim. Acta*, 1948, **31**, 818). This sequence of changes may be formulated by the partial formulæ (I)—(III).



(I.) (II.) (III.)

The reactions described above definitely established the presence of a *gem*-dimethyl group in a position vicinal to the hydroxyl group. This grouping is analogous to that observed in the case of the pentacyclic triterpenes (*idem*, *ibid.*, 1945, **28**, 767, 942, 1628). Furthermore, the formation of hydroxymethylenelanostenone from lanostenone—the latter, on oxidation with alkaline hydrogen peroxide gave a dibasic acid, $C_{30}H_{50}O_4$ (Ruzicka, Rey, and Muhr, *ibid.*, 1944, **27**, 472)—necessitates that a methylene group be placed α to the alcoholic hydroxyl group. It is clear from this evidence that the secondary hydroxyl group is flanked on one side by a *gem*-dimethyl group and on the other by a methylene group, *viz.*, $\cdot CMe_2 \cdot CH(OH) \cdot CH_2 \cdot$. The dibasic acid, $C_{30}H_{50}O_4$, when heated, lost carbon dioxide and cyclised, to give the norketone, $C_{29}H_{48}O$, and from this it follows that the hydroxyl group is present in a ring system which is probably six-membered (Ruzicka *et al.*, *locc. cit.*).

The reactive ethylenic linkage of lanostadienol has been shown to be present in an exocyclic *iso*propylidene group, since oxidative fission of lanostadienol and its derivatives gave acetone together with non-volatile acidic oxidation products (Dorée and Garratt, *J. Soc. Chem. Ind.*, 1933, **52**, 355T; Wieland and Benend, *Z. physiol. Chem.*, 1942, **274**, 215; Ruzicka, Rey, and Muhr, *loc. cit.*). On the basis of these results, the partial formula (IV) may be put forward for lanostadienol.



(IV.)

The conclusions summarized in the foregoing section are firmly based on experimental evidence, which is incapable of other interpretations. However, there exist in the literature two apparent discrepancies which are irreconcilable if the above facts are correct, and hitherto these have received no adequate explanation; first, the isolation by Marker, Wittle, and Mixon (*loc. cit.*) of a monobasic acid, $C_{25}H_{46}O_2$, on chromic acid oxidation of lanostadienyl or lanostenyl acetate; secondly, the isolation by Wieland and Joost (*loc. cit.*) of a monobasic acid

* Part XI, *J.*, 1951, 834. † The prefix A-nor denotes contraction of ring A.

formulated as $C_{27}H_{40}O_2$ (or less probably $C_{26}H_{38}O_2$ or $C_{28}H_{42}O_2$) on oxidation of lanostenol. We have reinvestigated these results with the hope of throwing further light on the problem as a whole, and we now record our own results and interpretations. These elucidate these inconsistencies and make possible the general acceptance of the partial formula for lanostadienol given above.

Marker, Wittle, and Mixon (*J. Amer. Chem. Soc.*, 1937, **59**, 1368) oxidised lanostadienyl and lanostenyl acetate with chromic acid under conditions analogous to those used for the oxidation of *epi*cholestanyl acetate to androsterone. No volatile products were obtained, but from the acid fraction a monobasic acid, m. p. 81° (methyl ester, m. p. 67°), formulated as $C_{31}H_{46}O_2$, was isolated. This was reported to be unchanged by boiling acetic anhydride, to be inert to ketonic reagents, and to be saturated to bromine in acetic acid, even at 60°. The neutral fraction obtained in the oxidation gave no semicarbazone and was not investigated further.

Basing their conclusions on the assumption that the original acetoxy-group was present in a terminal ring, Marker *et al.* came to the following conclusions. " If the acetate group is in the first ring of these compounds, then the active double bond must also be in that ring to lose five carbon atoms and give a saturated monocarboxylic acid identical to [*sic*] that formed from dihydrolanosteryl acetate. Getting a monocarboxylic acid from this ring system could be explained by a mechanism similar to that of the opening of ring B of ergosterol by ultra-violet light to give calciferol."

The experimental results of Marker *et al.* (*loc. cit.*) were apparently confirmed in subsequent work by Bellamy and Dorée (*J.*, 1941, 172), who found that oxidation of lanostadienol under the conditions used by Marker for the oxidation of the acetate gave a substance, m. p. 81—82° (ethyl ester, m. p. 64°), which was apparently identical with that described by the American workers. The interpretation of these results is at direct variance with more recent investigations as to the relative positions of the reactive olefinic linkage and the hydroxyl group.

Accordingly, we repeated the oxidation of lanostadienyl acetate under the conditions laid down by Marker *et al.* and by the modified method of Bellamy and Dorée (*locc. cit.*) and obtained a yellow amorphous product, from ethyl acetate, having m. p. *ca.* 80°, but giving analyses in fair agreement with the formula $C_{29}H_{42}O_4$. All attempts to obtain the acid, its salts, or its methyl ester in a crystalline form proved fruitless. However, we found that the amorphous oxidation product on treatment with 2% ethanolic potassium hydroxide gave a deep red solution which on being heated for 2 hours developed an emerald-green colour (reversal of these changes occurring on cooling). From the alkaline solution was isolated an acid, in moderate and variable yields, separating from ethyl acetate in fine yellow needles, m. p. 205—206°, $[\alpha]_D$ 104°; this gave analytical figures, after prolonged drying in a high vacuum at 140°, in good agreement with the formulation $C_{27}H_{40}O_5$. A determination of the equivalent of this substance by electrometric titration showed it to be a monobasic acid. The ultra-violet absorption spectra showed a maximum at 275 mμ. (log ε 4·03), which indicated the presence of an unsaturated 1 : 4-diketo-system similar to that present in diketolanostenyl acetate (Ruzicka *et al.*, *Helv. Chim. Acta*, 1944, **27**, 472). The fifth oxygen atom was shown as follows to be present in the secondary alcoholic group of the parent lanostadienol. Benzoylation gave a substance which after prolonged drying in a high vacuum had m. p. 218—220°, $[\alpha]_D$ 112°, and gave an analysis agreeing with $C_{34}H_{44}O_6$. This was identical with that obtained by the oxidation of lanostadienyl benzoate. Alkaline hydrolysis regenerated the original substance, m. p. 205—206°; mild oxidation of this substance with chromic acid gave a triketo-acid, isolated as the methyl ester, m. p. 149—150°, $[\alpha]_D$ +172°. These results confirm the conclusion that the yellow substance, $C_{27}H_{40}O_5$, m. p. 205—206°, is a hydroxydiketo-monobasic acid.

Our experimental findings are readily explained if Marker's primary oxidation product retains the acetoxy-group of lanostadienyl acetate and fission of the exocyclic *iso*propylidene group has taken place with simultaneous oxidation, to keto-groups, of the two methylene groups vicinal to the inert ethylenic bond; the subsequent action of the alkali would be a simple hydrolysis of the acetoxy-group. This interpretation was confirmed by the determination of the equivalent weight of Marker's product, which indicated the presence of both an acetoxy- and a carboxyl group. The yields of the hydroxydiketo-acid (diketotrisnorlanostenolic acid) from lanostadienyl acetate are not good (20—30% at the best), probably owing to further oxidation of the nucleus (*e.g.*, fission of ring A) to give more complex non-crystalline acidic products, which we are now studying.

Oxidation of lanostenyl acetate under the conditions used by Marker gave a small acidic fraction from which the diketotrisnorlanostenolic acid could be obtained. Although we have carried out this oxidation many times during the past eight years, the major oxidation product

was always diketolanostenyl acetate; since this may be partly extracted with strong alkali from ether, it may account for the large " acidic " fraction reported by Marker. We think that our own observations render unnecessary the premises of Marker *et al.* to account for the formation of their acidic degradation product.

The diketotrisnorlanostenolic acid described above is identical with an acid obtained by Wieland and Joost (*loc. cit.*) by chromic acid oxidation of cryptostadienyl benzoate, followed by hydrolysis. This acid was formulated by Wieland as $C_{28}H_{42}O_5$, although the analytical data quoted agree equally well with the alternative formulation $C_{27}H_{40}O_5$. A table of comparison of the two series of oxidation products is shown below and leaves little doubt as to their identity,

	Cryptostadienol.	Lanostadienol.	
	M. p.	M. p.	$[\alpha]_D$.
Benzoyloxy-acid	210—220°	218—220°	+112°
Hydroxy-acid	201—203	205—206	+104
Keto-acid (methyl ester)	154—155	149—150	+172

although Wieland and Joost (*loc. cit.*) gave no values for the specific rotations. Cryptosterol has been shown to be identical with lanostadienol (Ruzicka *et al.*, *Helv. Chim. Acta*, 1945, **28**, 759; for further evidence see McGhie, Ph.D. Thesis, London, 1947), and this provides further confirmation of the identity of the two triterpenes.

Our explanation of the mode of formation of these acids has been directly confirmed as follows. Ozonolysis of lanostadienol followed by methylation with diazomethane gave methyl trisnorlanostenolate (Wieland and Joost; Ruzicka, Rey, and Muhr, *locc. cit.*), which was oxidised with chromic acid to methyl diketotrisnorlanostenonate, identified by comparison with the ester obtained from diketotrisnorlanostenolic acid.

Wieland and Joost (*loc. cit.*) have also reported the oxidation of lanostenol by chromic acid to a crystalline monobasic acid, m. p. 195°, to which the formula $C_{27}H_{40}O_2$ (or less probably $C_{26}H_{38}O_2$) was assigned. They interpreted their results (with caution) on the assumptions that, in the production of the monobasic acid, the original hydroxyl group had been lost, that this hydroxyl group was present in a side chain, and that one of the four rings of the tetracyclic system had become aromatic. Since, as a result of their experiments on lanostenyl hydrochloride, they had concluded that the reactive double bond of lanostadienol is $\beta\gamma$ to the alcoholic hydroxyl group, it appeared likely that *both* the reactive double bond and the hydroxyl group were present in the side chain. This conclusion is in direct opposition to that of later workers.

A repetition of Wieland-and Joost's investigations resulted in the isolation of a substance, $C_{30}H_{50}O_4$, m. p 195°, $[\alpha]_D$ +87° (Wieland and Joost give no value for the specific rotation), the nature of which as a dibasic acid was disclosed by its equivalent weight and by preparation of a dimethyl ester which, although it could not be obtained crystalline, contained two methoxyl groups (Zeisel). Although our experiments were hampered by very poor yields of this acid, we were able to confirm its identity with a dibasic acid isolated by Ruzicka *et al.* (*Helv. Chim. Acta*, 1944, **27**, 472) by oxidation of hydroxymethylenelanostenone. The formation of the dibasic acid in the oxidative degradation of lanostenol is easily explained by cleavage of the terminal hydroxyl-bearing ring, thus :

We are of the opinion that a similar misinterpretation of experimental results exists in regard to the constitution of the acidic oxidation products obtained from the acetate of the closely related tetracyclic triterpene alcohol, euphadienol. Dupont, Dulon, and Vilkas (*Bull. Soc. chim.*, 1949, **16**, 809) have reported the oxidation of euphadienyl acetate with potassium permanganate to give a monobasic acid, $C_{27}H_{40}O_5$, m. p. 231—231·5°, $[\alpha]$ 14·2°. The corresponding methyl ester had m. p. 160·5—161°, 167·5—169·5°, and 190—190·5° depending on the solvent used for crystallisation. The acid was stated to possess an ultra-violet absorption maxima at 270 mμ. (log ε 3·89), which is very close to that of diketotrisnorlanostenolic acid, $\lambda_{max.}$ 275 mμ. (log ε 4·03). The acid was reported to be unaffected by acetic anhydride, to give no quinoxaline derivative with *o*-phenylenediamine, and to form an oxime, m. p. 207·5—208·5°. In a subsequent communication, Dupont *et al.* (*ibid.*, p. 813) stated that further oxidation of the acid, $C_{27}H_{40}O_5$, gave the nor-acid $C_{26}H_{38}O_5$, m. p. 191·5—192° (methyl ester, m. p. 140·5—141·5°). This

acid was also obtained from the mother-liquors obtained in the preparation of euphadienone by oxidation of euphadienol.

On the other hand, Krusi (*J.*, 1950, 2864) has reported that euphadienyl acetate, on dehydrogenation with *N*-bromosuccinimide followed by oxidation with chromic acid, gave an acid, isolated as the methyl ester, which on subsequent hydrolysis gave the acid, m. p. 233—234° (after sublimation, m. p. 231—233°); the methyl ester had m. p. 185—186°. The acid was assigned the molecular formula, $C_{25}H_{36}O_5$, and Krusi interpreted his results on the basis of this formula. It should be noted that Krusi assumed that in the course of dehydrogenation with *N*-bromosuccinimide a conjugated diene system had been formed, the new double bond being in conjugation with that originally present in the *iso*propylidene group, and that on oxidation it was this new double bond which was ruptured. The evidence for such a conjugated

$C_{19}H_{29}$ { $-CH_2\cdot CH_2\cdot CH{:}CMe_2$; $-CH_2\cdot C{:}C\cdot CH_2-$; $>CH\cdot OH$ } $C_{19}H_{29}$ { $-CH{:}CH\cdot CH{:}CMe_2$; $-CH_2\cdot C{:}C\cdot CH_2-$; $>CH\cdot OH$ } $C_{19}H_{29}$ { $-CO_2H$; $-CO\cdot C{:}C\cdot CO-$; $>CH\cdot OH$ }

system was the ultra-violet absorption spectra which showed maxima at 232 mμ. (log ε = 3·50), 238·5 mμ. (log ε = 3·52), and 247 mμ. (log ε = 3·33), these values being very similar to those reported by Bellamy and Dorée (*J.*, 1941, 176) for γ-lanostadienyl acetate, with maxima at 236, 244, and 250 mμ., which is known to contain a nuclear system of conjugated double bonds. It appears to us that Krusi ignored the alternative possibility that the introduction of a conjugated system could have involved the inert nuclear double bond; furthermore, since Dorée, McGhie, and Kurzer (*J.*, 1949, 570) have reported that dehydrogenation of lanostenyl acetate with *N*-bromosuccinimide gave γ-lanostadienyl acetate identical with that described by Bellamy and Dorée (*loc. cit.*), and this reaction must of necessity involve the endocyclic double bond to produce a conjugated system (cf. Birchenough and McGhie, *J.*, 1949, 2038), this possibility seems more plausible to us. Further, oxidation of γ-lanostadienyl acetate with chromic acid is known to yield diketolanostenyl acetate (Muhr, Thesis, Zurich, 1945; Cavalla and McGhie, *J.*, 1951, 834) showing an absorption maximum in the ultra-violet region at 275 mμ. (log ε = 3·94), which is very close to the values reported by Krusi and by Dupont *et al.* [$\lambda_{max.}$ 272 mμ. (log ε = 3·99) and 270 mμ. (log ε = 3·89), respectively] for their acid oxidation products.

It appears that the alternative explanation, which was not considered by Krusi, is the more probable, especially as the length of the side chain in the tetracyclic triterpenes, lanostadienol and euphadienol, is not known with certainty. Accordingly, we suggest that dehydrogenation with *N*-bromosuccinimide involves the introduction of a nuclear conjugated system $\cdot CH{:}\overset{|}{C}\cdot\overset{|}{C}{:}CH\cdot$ (cf. Dorée *et al.*, *loc. cit.*) which on further oxidation leads to the 1 : 4-unsaturated diketo-group situated in the nucleus and the simultaneous fission of the *iso*propylidene side chain resulting in the formation of the carboxyl group. On this hypothesis, Krusi's acidic oxidation product, and that of Dupont *et al.*, may both be formulated on the basis of a parent monobasic diketotrisnor-acid, $C_{27}H_{40}O_5$ (cf. diketotrisnorlanostenolic acid), the fifth oxygen atom being present in the secondary alcoholic hydroxyl group. The following table summarises the results of Krusi and of Dupont *et al.*

	Dupont *et al.*		Krusi.
	M. p.	[α].	M. p.
Acid	231—231·5°	+14·2°	233—234°
	245·5—246·5 †	+22·9	231—233 ‡
Methyl ester	160·5—161	+13·0	170—171
	167·5—169·5 *		168—170 ‡
	190—190·5 †		
Methyl ester of hydrolysed product			185—186

* Crystallised from acetic anhydride. † Crystallised from aqueous alcohol.
‡ Resublimed.

A comparison of these two products shows their close similarity and it appears on this evidence that the two acids are identical although certain discrepancies have still to be explained. For instance, Krusi isolated his acid by hydrolysis of the methyl acetoxy-ester, whereas Dupont *et al.* obtained theirs by direct oxidation of euphadienyl acetate. It is possible that in Dupont's work hydrolysis occurred during isolation, or that Krusi incompletely hydrolysed his compound.

The reported oxidation by Dupont of his acid to the nor-acid, $C_{28}H_{38}O_5$, probably involves a simple oxidation of the secondary alcoholic group to give a triketo-acid, $C_{27}H_{36}O_5$ (cf. formation of methyl diketotrisnorlanostenonate from diketotrisnorlanostenolic acid). In favour of our tentative suggestions, it should be noted that the analytical data recorded by both groups of workers may be fairly satisfactorily accommodated on our assumption that the parent acid possesses the molecular formula, $C_{27}H_{40}O_5$. We hope soon to report experiments designed to prove conclusively the correctness of this assumption.

Experimental.

All m. p.s are uncorrected. Specific rotations were measured in chloroform solution at 20°. Aluminium oxide used in chromatographic work was from Mesrs. Peter Spence Ltd. (Grade " O "). All analyses are by Drs. Weiler and Strauss, Oxford.

Oxidation of Lanostadienyl Acetate according to Marker et al. (loc. cit.).—Lanostadienyl acetate (5 g.) in glacial acetic acid (100 ml.) was treated at 90° with chromic acid (10 g.) in 90% acetic acid (50 ml.), added dropwise during 3 hours with constant mechanical stirring; this temperature was maintained for a further 3 hours, and the mixture then set aside at room temperature overnight. The excess of chromic acid was destroyed by addition of ethyl alcohol, and the solution evaporated to dryness under reduced pressure. The residue was dissolved in ether, and the ethereal solution washed with water and sodium hydrogen carbonate solution, then extracted with 10% sodium hydroxide solution. The alkaline extracts were washed with ether to remove any neutral fraction and acidified to Congo-red with dilute hydrochloric acid, and the precipitated solids extracted with ether. The ethereal layer was washed with water and dried (Na_2SO_4), and the solvent removed to leave a dark yellow gum (4 g.). This was dissolved in ethyl acetate, and the solution on long storage deposited a yellow amorphous product (3 g.), m. p. 80—81° (Found: C, 72·2; H, 9·1. Calc. for $C_{33}H_{44}O_3$: C, 79·3; H, 12·3. Calc. for $C_{35}H_{42}O_6$: C, 71·6; H, 8·6%). Attempts to obtain crystalline salts were unsuccessful; methylation, using diazomethane, followed by chromatography gave no crystalline material.

Oxidation of lanostenyl acetate (5 g.) with chromic acid, under the same conditions as for lanostadienyl acetate, gave a small non-crystalline acid fraction (0·2 g.), the main product being the neutral diketo-lanostenyl acetate, m. p. 158—159°, $[\alpha]_D$ +91·6° (*c*, 0·024), identical with an authentic specimen.

Modified Method for the Preparation of Marker's Oxidation Product.—Lanostadienyl acetate (5 g.) in glacial acetic acid (300 ml.) was treated at 90° with a solution of chromic acid (2·5 g.) in 90% acetic acid (50 ml.) added dropwise, with stirring, during an hour. Stirring was continued at the same temperature for a further hour, after which the mixture was poured on crushed ice, and the excess of chromic acid destroyed by addition of sulphurous acid. The precipitated solid was filtered off and taken up in ether, and the ethereal solution extracted with 10% sodium hydroxide solution. The alkaline extracts were washed with ether and acidified to Congo-red with dilute hydrochloric acid, and the precipitated acid was extracted with ether in the usual manner to give a yellow gum (3 g.) on the removal of the ether. Crystallisation of this product from ethyl acetate gave an amorphous yellow material, m. p. 79—81°, identical with that prepared from lanostadienyl acetate according to Marker's procedure (Found: equiv., 400, 420. Calc. for $C_{35}H_{42}O_6$: equiv., 486. An acetyl determination showed the presence of 0·95 acetoxy-group].

Treatment of Marker's Oxidation Product with Alkali.—The oxidation product (2 g.) was heated under reflux with a 2% solution of alcoholic potassium hydroxide (200 ml.) for 3 hours, and the mixture then poured into water and extracted with ether in the usual manner. Removal of the ether gave no residue. The alkaline solution was acidified to Congo-red with dilute hydrochloric acid, and the precipitated solids were extracted with ether, the ethereal layer was washed and dried (Na_2SO_4), and the solvent removed to leave a pale yellow solid, which, crystallised from ethyl acetate, had m. p. 201—203°. Two further crystallisations from ethyl acetate gave pale yellow needles of *diketotrisnorlanostenolic acid*, m. p. 205—206°, $[\alpha]_D$ +104° (*c*, 0·026) (Found: C, 73·2, 73·1; H, 8·9, 8·9%; equiv., 440, 438. $C_{27}H_{40}O_5$ requires C, 73·6; H, 9·0%; equiv., 444).

Methylation of this acid by means of diazomethane, and crystallisation of the product from aqueous methanol, gave the *methyl* ester, m. p. 175—176° (Found: C, 73·0; H, 9·2. $C_{28}H_{42}O_5$ requires C, 73·3; H, 9·2%).

Benzoylation. Diketotrisnorlanostenolic acid (1 g.) in pyridine (25 ml.) was treated with benzoyl chloride (1·5 ml.) at room temperature for 16 hours. The mixture was poured into water, filtered, washed with dilute hydrochloric acid, and crystallised from acetic acid, giving a product, m. p. 200°; two further crystallisations from the same solvent gave yellow prisms of *diketotrisnorlanostenolic acid benzoate*, m. p. 218—220°, $[\alpha]_D$ +112° (*c*, 0·04) (Found: C, 74·3; H, 8·2. $C_{34}H_{44}O_6$ requires C, 74·5; H, 8·0%). Methylation of this acid by diazomethane, and crystallisation from ethyl acetate–methanol, gave the *methyl* ester, m. p. 197—199° (Found: C, 75·0; H, 8·0. $C_{35}H_{46}O_6$ requires C, 74·7; H, 8·2%).

Oxidation. Diketotrisnorlanostenolic acid (0·25 g.) in glacial acetic acid (25 ml.) was treated at 45—50° with a solution of chromic acid (0·15 g.) in 90% acetic acid (5 ml.). The temperature was maintained for 3 hours, and the excess of chromic acid was then destroyed with sulphurous acid, and the mixture poured into ice-water. The precipitated solids were extracted with ether and worked up in the usual manner, to give a yellow oil which could not be crystallised. This was taken up in ether and treated with an ethereal solution of diazomethane for 4 hours. The product on removal of the ether was crystallised repeatedly from methanol, giving yellow needles of *methyl diketotrisnorlanostenonate*, m. p. 149—150°, $[\alpha]_D$ +172° (*c*, 0·036) (Found: C, 73·8; H, 8·9. $C_{28}H_{40}O_5$ requires C, 73·7; H, 8·8%).

Hydrolysis of this ester with 3% alcoholic potassium hydroxide gave only intractable yellow gums.

Oxidation of Lanostadienyl Benzoate.—Lanostadienyl benzoate (2 g.) in glacial acetic acid (300 ml.) was stirred mechanically at 80—90° while a solution of chromic acid (4 g.) in 90% acetic acid (60 ml.) was added during an hour. At the end of this time the suspended benzoate had passed completely into solution; the temperature was maintained at 80—90°, with stirring, for a further 2 hours. The mixture was then poured into ice-water, the excess of chromic acid destroyed with sulphurous acid, and the precipitate filtered off, washed, and air-dried, and then dissolved in ether. The ethereal layer was washed and separated into a neutral and an acidic fraction in the usual manner. The oily neutral product (0·1 g.) was not further investigated. The acid fraction, crystallised from acetic acid, had m. p. 210°, raised by two further crystallisations to 218—220°, $[\alpha]_D$ +112°. This was diketotrisnorlanostenolic acid benzoate, identical with that prepared by benzoylation of diketotrisnorlanostenolic acid (Found: C, 74·5, 74·3; H, 8·1, 8·1%).

Ozonolysis of Lanostadienol.—Lanostadienol (1 g.), dissolved in absolute chloroform (25 ml.), was treated with a dry stream of ozonised oxygen (3% v/v) for 3 hours. The solvent was removed under reduced pressure, and the glassy residue boiled under reflux with water (200 ml.) for 1 hour; 75 ml. of the liquid were then distilled into a solution of 2 : 4-dinitrophenylhydrazine in 10% hydrochloric acid. The yellow precipitate was filtered off and crystallised from alcohol, giving orange needles of acetone 2 : 4-dinitrophenylhydrazone, m. p. 128° (40%) (Found: C, 45·4; H, 4·3; N, 23·3. Calc. for $C_9H_{10}O_4N_4$: C, 45·4; H, 4·2; N, 23·5%). The non-volatile residue was separated into a neutral and an acidic fraction. The latter was worked up in one of two ways. Repeated crystallisation from acetic acid gave trisnorlanostenolic acid, m. p. 257—259°, $[\alpha]_D$ +52° (*c*, 0·065) (Found: C, 77·7; H, 10·7. Calc. for $C_{27}H_{44}O_3$: C, 77·8; H, 10·6%).

Methylation (diazomethane) gave the methyl ester, m. p. 154—155° (Found: C, 78·0; H, 10·9. Calc. for $C_{28}H_{46}O_3$: C, 78·1; H, 10·8%). Alternatively, the product was methylated with diazomethane, this product was adsorbed on alumina (12 × 1·3 cm.) from benzene–hexane (1 : 1; 100 ml.), and the column eluted with the same solvent mixture (250 ml.). Removal of the solvent and crystallisation of the residue from chloroform–methanol gave methyl trisnorlanostenolate, m. p. 154—155°. Hydrolysis gave the acid, m. p. 257—259°, identical with that obtained above.

Oxidation of Methyl Trisnorlanostenolate.—Oxidation of methyl trisnorlanostenolate, by the method described above for the oxidation of diketotrisnorlanostenolic acid, gave methyl diketotrisnorlanostenonate, m. p. 149—150°, $[\alpha]_D$ +170° (*c*, 0·04), identical with an authentic specimen. This ester could be obtained directly from lanostadienol in poor yield by following Bellamy and Dorée's conditions (*J.*, 1941, 172). The acidic oxidation product was methylated with diazomethane, and the crude methyl ester dissolved in benzene (150 ml.) and adsorbed on a column of alumina (12 × 1·3 cm.), and the column eluted with benzene (200 ml.). Removal of the solvent and crystallisation of the residue from methanol gave methyl diketotrisnorlanostenonate, identical with that prepared above.

Oxidation of Lanostenol.—Lanostenol (4·8 g.) in "AnalaR" acetic acid (400 ml.) was treated at 80—85° with a solution of chromic acid (8 g.) in 90% acetic acid (160 ml.), added dropwise during 2 hours with mechanical stirring. The mixture was poured on crushed ice, the excess of oxidant destroyed with sulphurous acid, and the solution extracted with ether. The ethereal layer was washed repeatedly with 5% sodium hydroxide solution until the alkaline washings were tinged faintly with red (this indicated that all the acetic acid had been removed). Further extraction gave a reddish-brown alkaline solution, from which, when kept overnight, crystals of the sodium salt separated. These were filtered off, suspended in ether, and added to dilute hydrochloric acid, in which they dissolved; the ethereal solution was washed and dried (Na_2SO_4), and the solvent removed to leave a small solid residue (50 mg.), which on crystallisation from acetic acid gave a colourless solid, m. p. 195—196°, $[\alpha]_D$ +87° (*c*, 0·05) (Found: C, 76·0; 76·1; H, 10·6, 10·5%; equiv., 240, 244. Calc. for $C_{30}H_{46}O_4$: C, 75·9; H, 10·6%; equiv., 237). Methylation in the usual manner gave a methyl ester as an uncrystallisable oil [Found: (Zeisel's method), OMe, 11·8, 11·6. Calc. for $C_{31}H_{48}O_4$: OMe, 12·28%].

This acid was identical with the dicarboxylic acid obtained by oxidation of hydroxymethylenelanostenone (Ruzicka *et al.*, *Helv. Chim. Acta*, 1944, **27**, 472).

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546. *Acetals of Glutardialdehyde. The Preparation of* 1 : 1 : 5 : 5-*Tetraethoxy-* and 1 : 1 : 5 : 5-*Tetra*-n-*butoxy-pentanes.**

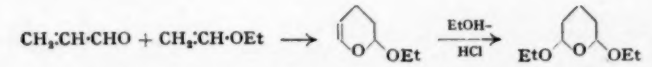
By R. H. Hall and B. K. Howe.

Reaction of glutardialdoxime with ethyl nitrite and treatment of the product with ethanolic hydrogen chloride and calcium chloride yielded, contrary to Baudart's findings (*Bull. Soc. chim.*, 1944, **11**, 336), only a minor amount of 1 : 1 : 5 : 5-tetraethoxypentane, the main products being ethyl 4 : 4-diethoxybutane-1-carboxylate and 2 : 6-diethoxytetrahydropyran. The last was synthesized from acraldehyde and ethyl vinyl ether, by way of 6-ethoxy-Δ^2-dihydropyran, and was converted into 1 : 1 : 5 : 5-tetraethoxypentane by reaction with excess of ethanolic hydrogen chloride. 1 : 1 : 5 : 5-Tetra-*n*-butoxypentane was similarly obtained from acraldehyde and *n*-butyl vinyl ether by way of 6-*n*-butoxy-Δ^2-dihydropyran and 2 : 6-di-*n*-butoxytetrahydropyran.

The only reported synthesis of an acetal of glutardialdehyde is that of the bisdiethyl acetal, 1 : 1 : 5 : 5-tetraethoxypentane (Baudart, *Bull. Soc. chim.*, 1944, **11**, 336), in which pyridine is converted into glutardialdoxime by way of 1 : 4-dihydropyridine by Shaw's method (*J.*, 1925, 215; 1937, 301): the dioxime is decomposed with ethyl nitrite at 0°, and the unstable glutardialdehyde produced is converted directly into its bisdiethyl acetal. No elementary analyses are recorded, however, for the product.

An acetal of glutardialdehyde was required for another investigation and the preparation of the bisdiethyl acetal was therefore attempted along the above lines. The decomposition of the dioxime with ethyl nitrite, however, invariably proved troublesome. Initiation of the reaction was difficult and seemed to depend very much on the moisture content of the oxime. When the latter had been dried in an oven (at 105°) the reaction was always more difficult to start than when the dioxime was air-dried, even though the melting point was not altered. Addition of a small amount of water and warming to 5—10° proved helpful in ensuring a fairly rapid commencement of the reaction, which then proceeded satisfactorily at about 0°. Treatment of the crude dialdehyde solution thus obtained with ethanolic hydrogen chloride and calcium chloride and isolation of the products, however, always gave rise to two main fractions, the amounts and proportions of which were very variable.

The lower-boiling fraction was shown to be the hitherto unknown 2 : 6-diethoxytetrahydropyran by elementary analyses, molecular-weight determinations, absence of aldehyde, ester, lactone, and hydroxyl groups, determination of oximation equivalent, conversion into glutardialdehyde bis-2 : 4-dinitrophenylhydrazone, and conversion into 1 : 1 : 5 : 5-tetraethoxypentane by excess of ethanolic hydrogen chloride. Its infra-red absorption spectrum was also compatible with the above formulation. It was synthesized by treating acraldehyde with ethyl vinyl ether under pressure (cf. B.P. Appln. 14678/48 to N.V. de Bataafsche Petroleum Maatschappij), to form 6-ethoxy-Δ^2-dihydropyran (subsequently prepared by Longley and Emerson, *J. Amer. Chem. Soc.*, 1950, **72**, 3079) which was then treated with ethanol in the presence of hydrogen chloride:



The higher-boiling fraction was shown to be ethyl 4 : 4-diethoxybutane-1-carboxylate containing a little 1 : 1 : 5 : 5-tetraethoxypentane. Alkaline hydrolysis, followed by acid hydrolysis, yielded 3-formylpropane-1-carboxylic acid, which was characterized as its 2 : 4-dinitro- and *p*-nitro-phenylhydrazones, identical with synthetic samples obtained from authentic 3-formylpropane-1-carboxylic acid. The latter was prepared from *cyclo*pentanone by Treibs's method (*Ber.*, 1939, **72**, 1194). A little glutardialdehyde, which was characterized as its bis-2 : 4-dinitrophenylhydrazone, was also formed in the acid hydrolysis.

The use of alkyl nitrites or nitrous fumes to convert oximes into the corresponding free carbonyl compounds, particularly in circumstances where mineral acids cannot be employed,

* Part of this work is described in B.P. Appln. 11,261/50.

is well known (Claisen and Manasse, *ibid.*, 1888, **21**, 2176; 1889, **22**, 526, 530; Harries, *ibid.*, 1901, **34**, 1488; von Braun and Danziger, *ibid.*, 1913, **46**, 110) but there appear to be no references to the formation of esters as by-products in this type of reaction. The ethyl 4 : 4-diethoxybutane-1-carboxylate may have arisen from partial oxidation of the intermediate glutardialdehyde, followed by esterification and acetal formation, but this oxidation could hardly have been due to atmospheric oxygen since a nitrogen atmosphere was used initially and nitrous oxide was evolved throughout the reaction of the oxime with the ethyl nitrite, thus providing a cover of inert gas.

The 2 : 6-diethoxytetrahydropyran, which may be regarded as a cyclodehydration product of the bishemiacetal of glutardialdehyde, probably arose during acetal formation from the crude glutardialdehyde solution with ethanolic hydrogen chloride and calcium chloride, thus demonstrating the relative ease of formation and stability of this ring structure. Further evidence for the stability of the compound was provided by the recovery of some unchanged material when it was treated at room temperature with a large excess of ethanol containing hydrogen chloride to convert it into the desired 1 : 1 : 5 : 5-tetraethoxypentane. The latter reaction enabled the tetraethoxy-compound to be prepared either from glutardialdoxime by a modified version of Baudart's method or from acraldehyde and ethyl vinyl ether by way of 6-ethoxy-Δ^2-dihydropyran. The latter route is more attractive as the overall yields are higher, and, as it is obviously possible to proceed in one step from the 6-ethoxy-Δ^2-dihydropyran to the tetraethoxy-compound, 1 : 1 : 5 : 5-tetraethoxypentane can thus be prepared in two stages from acraldehyde and ethyl vinyl ether.

More recently the route from acraldehyde has been used for the preparation of 1 : 1 : 5 : 5-tetra-*n*-butoxypentane. 6-*n*-Butoxy-Δ^2-dihydropyran was prepared from acraldehyde and *n*-butyl vinyl ether by the method of Longley and Emerson (*loc. cit.*) and treated with a molecular proportion of *n*-butanol in the presence of acid at 30—40°, to give 2 : 6-di-*n*-butoxytetrahydropyran in 73% yield. Ring scission with excess of butanol containing hydrogen chloride at 40° for 2 days was incomplete, about one-half of the dibutoxy-compound being recovered unchanged, but 1 : 1 : 5 : 5-tetra-*n*-butoxypentane was isolated from the product in 31% yield (65% after allowance for recovered starting material).

Experimental.

M. p.s and b. p.s are uncorrected. Microanalyses are by Drs. Weiler and Strauss of Oxford.

Glutardialdoxime.—Glutardialdoxime, m. p. 175°, was prepared from pyridine by Shaw's method (*locc. cit.*). With ethanolic 2 : 4-dinitrophenylhydrazine hydrochloride solution it gave the bis-2 : 4-dinitrophenylhydrazone, as tiny brownish-yellow needles, m. p. 186—187° (from acetic acid) (Found : C, 44·85, 44·85; H, 3·35, 3·5; N, 24·3. Calc. for $C_{17}H_{16}O_8N_8$: C, 44·35; H, 3·5; N, 24·3%) (Shaw, *J.*, 1937, 301, gives m. p. 169—172°).

Reaction of Glutardialdoxime with Ethyl Nitrite (cf. Baudart, *loc. cit.*).—Glutardialdoxime (60 g.) and ethyl nitrite (80 g.; *Org. Synth.*, Coll. Vol. II, p. 204) were added alternately in small portions, as fast as reaction occurred, to a stirred mixture of 95% ethanol (17 c.c.), water (*ca.* 2 c.c.) and acetic acid (1·7 c.c.) which was kept under nitrogen and cooled from time to time in solid carbon dioxide–alcohol to keep its temperature at about 0°. The reaction started sluggishly after gentle warming to 5—10° but after a time proceeded quite readily at 0°. When all the reactants had been added the mixture was stirred until all the oxime had dissolved, ethanol (45 c.c.) containing anhydrous hydrogen chloride (1·5 g.) was added slowly, and then finely-powdered anhydrous calcium chloride (13·5 g.) was stirred in. The mixture was kept at 0° for 5 hours, then at room temperature for 48 hours, and extracted several times with light petroleum (b. p. 40—60°). The combined extracts were washed with water, dried (K_2CO_3), and evaporated *in vacuo*, and the oil obtained was distilled *in vacuo*, distillate (36 g.) of boiling-range 80—140°/12 mm. being collected. (The weight of crude material obtained in different experiments varied from 45 to 80% of the initial weight of glutardialdoxime.) Fractionation of this distillate through a jacketed glass column (25 × 1·5 cm.) packed with single-turn glass helices gave two main fractions : (A) 13 g., b. p. 79—80°/11 mm., n_D^{19} 1·4291, and (B) 15 g., b. p. 120—124°/13 mm., n_D^{20} 1·4220—1·4228.

Fraction (A) contained traces of an ester from which it was freed by refluxing it with alcoholic N-potassium hydroxide, dilution with water, ether-extraction, drying (Na_2SO_4), evaporation, and fractionation of the residual oil *in vacuo*. 2 : 6-*Diethoxytetrahydropyran* was obtained as a colourless liquid, b. p. 82°/13 mm., n_D^{20} 1·4290, d_4^{20} 0·9673 (Found : C, 62·15; H, 10·35%; *M* (cryoscopic in camphor), 181; oximation equiv., 91; $[M]_D^{20}$ 46·43. $C_9H_{18}O_3$ requires C, 62·05; H, 10·4%; *M*, 174; oximation equiv., 87; $[M]_D^{20}$ 46·50), which did not react with Tollens's reagent, with boiling alcoholic N-potassium hydroxide, or with hot acetic anhydride in pyridine. Its infra-red absorption spectrum confirmed the absence of functional groups other than C–O–C. On treatment with aqueous 2 : 4-dinitrophenylhydrazine hydrochloride solution it gave glutardialdehyde bis-2 : 4-dinitrophenylhydrazone, m. p. 186° (from acetic acid), undepressed on admixture with an authentic specimen.

Fraction (B) (sap. equiv., 233·5; oximation equiv., 193) consisted mainly (80—90%) of ethyl 4 : 4-diethoxybutane-1-carboxylate contaminated with a little 1 : 1 : 5 : 5-tetraethoxypentane. Hydrolysis of a sample (0·6 g.) of the fraction with boiling alcoholic N-potassium hydroxide solution

(10 c.c.) for 1 hour, neutralisation with N-sulphuric acid, and addition of aqueous 2 : 4-dinitrophenylhydrazine hydrochloride solution gave a precipitate which was separated into sodium hydrogen carbonate-soluble and -insoluble derivatives. The soluble derivative was *3-formylpropane-1-carboxylic acid* 2 : 4-*dinitrophenylhydrazone* and crystallised from 80—90% ethanol as small golden-yellow needles, m. p. 139—140° (after sintering at 125°) (Found : C, 44·75; H, 4·25; N, 18·8. $C_{11}H_{12}O_6N_4$ requires C, 44·6; H, 4·1; N, 18·9%), undepressed on admixture with an authentic specimen (Found : C, 44·45; H, 4·15%) prepared from 3-formylpropane-1-carboxylic acid which was synthesized from *cyclo*pentanone by Treibs's method (*loc. cit.*). The bicarbonate-insoluble derivative was glutardialdehyde bis-2 : 4-dinitrophenylhydrazone, m. p. 186° (from acetic acid), undepressed on admixture with an authentic specimen. Similar hydrolysis and treatment of the hydrolysate with *p*-nitrophenylhydrazine hydrochloride gave, as a bicarbonate-soluble derivative, 3-formylpropane-1-carboxylic acid *p*-nitrophenylhydrazone, golden-yellow crystals, m. p. 147—148°, from dilute ethanol (Harries and Tank, *Ber.*, 1908, **41**, 1708, give m. p. 148·5°). A mixed m. p. with synthetic 3-formylpropane-1-carboxylic acid *p*-nitrophenylhydrazone, prepared from authentic 3-formylpropane-1-carboxylic acid (see above), was also 147—148°.

Conversion of 2 : 6-*Diethoxytetrahydropyran into* 1 : 1 : 5 : 5-*Tetraethoxypentane.*—Ethanol (80 c.c.) containing anhydrous hydrogen chloride (2·4 g.) was added to 2 : 6-diethoxytetrahydropyran (21 g.), and the mixture was kept for 3 days at room temperature. Ethanolic sodium ethoxide was added cautiously to the resultant deep red solution until it was just alkaline and the excess of alkali was neutralised with carbon dioxide. The ethanol was distilled off and the residual oil was distilled *in vacuo*, distillate (22 g.) of boiling-range 90—145°/19 mm. being obtained. Fractionation of this distillate *in vacuo* through the column described above gave a small fore-run of unchanged starting material and then the desired 1 : 1 : 5 : 5-tetraethoxypentane as a colourless oil (9·9 g.), b. p. 133—134°/13 mm., n_D^{20} 1·4204 (Found : C, 62·6; H, 11·15%; oximation equiv., 128. Calc. for $C_{13}H_{28}O_4$: C, 62·85; H, 11·35%; oximation equiv., 124). Baudart (*loc. cit.*) gives b. p. 97—100°/3 mm., n_D^{25} 1·4232, d^{25} 0·9009. On treatment with aqueous-alcoholic 2 : 4-dinitrophenylhydrazine sulphate solution the tetraethoxy-compound gave glutardialdehyde bis-2 : 4-dinitrophenylhydrazone, m. p. 186—187° (from acetic acid), undepressed on admixture with an authentic specimen.

Modified Preparation of 1 : 1 : 5 : 5-*Tetraethoxypentane from Glutardialdoxime.*—Glutardialdoxime (120 g.) and ethyl nitrite (160 g.) were added alternately in small portions to a stirred, cooled mixture of 95% ethanol (33 c.c.), acetic acid (3·3 c.c.), and water (10 c.c.) as described above, the resultant solution was treated with ethanol (*ca.* 80 c.c.) containing anhydrous hydrogen chloride (4 g.), and anhydrous calcium chloride (27 g.) was added. The mixture was kept at 0° for a few hours, then at room temperature for two days, neutralised with ethanolic sodium ethoxide, made slightly acid with acetic acid, evaporated to a small bulk *in vacuo*, diluted with light petroleum (b. p. 40—60°) and water, and agitated. The aqueous layer was separated and extracted again with light petroleum, and the combined extracts were dried (K_2CO_3) and distilled. The distillate (91·5 g.) of boiling range 80—160°/13 mm. was mixed with a solution of potassium hydroxide (10 g.) in ethanol (100 c.c.) and water (10 c.c.), the mixture was refluxed for 1½ hours, almost neutralised with 0·2N-sulphuric acid (added cautiously with vigorous stirring), filtered, concentrated *in vacuo*, diluted with ether and water, and agitated, and the ethereal extract was separated. Evaporation of the dried (K_2CO_3) ethereal solution gave an oil which was dissolved in ethanol (500 c.c.) containing anhydrous hydrogen chloride (15 g.), the solution was kept at room temperature for 2—3 days, neutralised with ethanolic sodium ethoxide, filtered, concentrated *in vacuo*, and extracted with light petroleum (b. p. 40—60°), and the extracts were washed with water, dried (K_2CO_3), and evaporated. The residual oil was distilled and the distillate (68·9 g.), boiling range 90—140°/12 mm., was fractionated through the column described above, yielding 2 : 6-diethoxytetrahydropyran (9·1 g.), b. p. 80°/11·5 mm., n_D^{20} 1·4295, and 1 : 1 : 5 : 5-tetraethoxypentane (38·0 g.), b. p. 133°/12 mm., n_D^{20} 1·4201, d_4^{20} 0·9135 (Found : C, 63·2; H, 11·45%; $[M]_D^{20}$ 68·82. Calc. for $C_{13}H_{28}O_4$: C, 62·85; H, 11·35%; $[M]_D^{20}$, 68·81), as colourless liquids.

6-*Ethoxy*-Δ^2-*dihydropyran.*—A mixture of acraldehyde (112 g.), ethyl vinyl ether (144 g.), and quinol (2·4 g.) was heated, in portions, in sealed tubes at 185—195° for 2½—3 hours. The pale yellow products were combined and distilled *in vacuo*, distillate (207 g.) of boiling range 30—125°/125 mm. (mainly 84—90°/125 mm.) being collected; the residue was 28 g. The distillate was fractionated *in vacuo* through a helices-packed, jacketed, glass column (35 × 2 cm.), whereupon 6-ethoxy-Δ^2-dihydropyran, was obtained as a colourless liquid (147·7 g.), b. p. 66°/52 mm., n_D^{20} 1·4420 (Found : C, 65·8; H, 9·15. Calc. for $C_7H_{12}O_2$: C, 65·6; H, 9·45%) (Longley and Emerson, *loc. cit.*, subsequently found b. p. 42°/16 mm. n_D^{20} 1·4376). A test portion, on treatment with an alcoholic solution of 2 : 4-dinitrophenylhydrazine containing sulphuric acid, gave glutardialdehyde bis-2 : 4-dinitrophenylhydrazone, m. p. 185—186° (from acetic acid), undepressed on admixture with an authentic specimen.

2 : 6-*Diethoxytetrahydropyran.*—A mixture of 6-ethoxy-Δ^2-dihydropyran (128 g.) and ethanol (48 c.c.) was cooled to 0°, stirred, and treated cautiously with a solution of hydrogen chloride (0·63 g.) in absolute ethanol (10 c.c.). Heat was liberated and ice-cooling was employed. The mixture was kept at room temperature overnight, then made just alkaline by the cautious addition of concentrated ethanolic sodium ethoxide, and the excess of alkali neutralised with carbon dioxide. The product was distilled *in vacuo*, distillate of boiling-range 50°/50 mm. to 140°/12 mm. being collected; the residue was 12 g. Fractionation of the distillate through the column described above gave 2 : 6-diethoxytetrahydropyran (83·4 g.), b. p. 78—79°/11 mm., n_D^{20} 1·4297, d_4^{20} 0·9677, identical with that obtained above from glutardialdoxime.

2 : 6-*Di*-n-*butoxytetrahydropyran.*—Concentrated hydrochloric acid (0·5 c.c.; *d* 1·18) was added to a cooled, stirred mixture of *n*-butanol (29 g.) and 6-*n*-butoxy-Δ^2-dihydropyran (61 g.; Longley and Emerson, *loc. cit.*), and the resultant solution was kept at 30—40° for 22 hours. Addition of anhydrous sodium acetate (1 g.), followed by fractional distillation *in vacuo*, gave first unchanged butanol and then

the desired 2 : 6-*di*-n-*butoxytetrahydropyran* as a colourless mobile liquid (86 g., 73%), b. p. 126°/9 mm., n_D^{20} 1·4371 (Found: C, 68·1, 67·9; H, 11·7, 11·55. $C_{13}H_{26}O_3$ requires C, 67·8; H, 11·4%).

1 : 1 : 5 : 5-*Tetra*-n-*butoxypentane*.—Concentrated hydrochloric acid (3 c.c.; *d* 1·18) was added to a stirred mixture of *n*-butanol (185 g.) and 2 : 6-di-*n*-butoxytetrahydropyran (57·5 g.), and the resultant solution was kept at 40° for 2 days. The product was cooled, made slightly alkaline with a solution of sodium butoxide in butanol, and fractionated *in vacuo* to give: (i) butanol, (ii) unchanged 2 : 6-di-*n*-butoxytetrahydropyran (*ca.* 30 g.), and (iii) 1 : 1 : 5 : 5-*tetra*-n-*butoxypentane* (28·3 g.), b. p. 197·5—198·5°/9 mm., n_D^{20} 1·4343 (Found: C, 70·1, 70·15; H, 12·4, 12·2. $C_{21}H_{44}O_4$ requires C, 69·9; H, 12·3%).

Addition of a few drops of the tetrabutoxy-compound to aqueous-alcoholic 2 : 4-dinitrophenylhydrazine sulphate gave glutardialdehyde bis-2 : 4-dinitrophenylhydrazone, m. p. 186—187° (from acetic acid), undepressed on admixture with an authentic specimen.

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547. *Some Reactions of* 1 : 2-3 : 4-*Diepoxybutane.*

By W. F. BEECH.

The addition of amines, alcohols, phenols, and certain other reactive substances to 1 : 2-3 : 4-diepoxybutane has been studied and the structures of the principal addition products have been established. In general, the mode of addition is such as to produce 1 : 4-derivatives of butane-2 : 3-diol.

1 : 2-3 : 4-DIEPOXYBUTANE was first prepared by Przybytek (*Ber.*, 1884, **17**, 1093) who obtained a substance of molecular formula $C_4H_6O_2$, m. p. +15°, by treating an ethereal solution of 1 : 4-dichlorobutane-2 : 3-diol with solid potassium hydroxide. Griner (*Compt. rend.*, 1893, **117**, 555) later obtained the same substance by using the higher-melting form of 1 : 4-dibromobutane-2 : 3-diol in place of the dichloro-compound. On hydrolysis, the new compound gave *meso*erythritol, and with hydrogen chloride 1 : 4-dichlorobutane-2 : 3-diol was regenerated. Certain other addition reactions were investigated by Przybytek (*loc. cit.*; *Ber.*, 1887, **20**, 3237) but only in the case of aniline was the structure of the product demonstrated experimentally.

By treating the lower-melting form of 1 : 4-dibromobutane-2 : 3-diol with potassium hydroxide in ether, Griner obtained a second stereoisomeric form of 1 : 2-3 : 4-diepoxybutane, m. p. 4°. This substance yielded DL-erythritol on hydrolysis and gave the lower-melting form of 1 : 4-dibromobutane-2 : 3-diol on treatment with hydrobromic acid.

A convenient starting material for the preparation of both forms of 1 : 2-3 : 4-diepoxybutane is the higher-melting (53—54°) form of 1 : 4-dibromobut-2-ene. On hydrolysis, this compound gives but-2-ene-1 : 4-diol which, by bromination and subsequent treatment of the dibromo-compound with potassium hydroxide in ether, affords 1 : 2-3 : 4-diepoxybutane identical with that obtained by Przybytek (Prévost, *Compt. rend.*, 1926, **183**, 1292). Oxidation of 1 : 4-dibromobut-2-ene (m. p. 53—54°) with dilute potassium permanganate, however, yields the lower-melting form of 1 : 4-dibromobutane-2 : 3-diol from which Griner (*loc. cit.*) prepared the other stereoisomeric form of the diepoxide.

A further possible source of 1 : 2-3 : 4-diepoxybutane is the mixture of butadiene dichlorohydrins obtained by Evans and Owen (*J.*, 1949, 239) by the action of hypochlorous acid upon 2-chlorobut-3-en-1-ol. As the latter compound is readily prepared from the commercially available 3 : 4-epoxybut-1-ene (Kadesch, *J. Amer. Chem. Soc.*, 1946, **68**, 44), it seemed possible that reasonably large amounts of the diepoxide might be obtained from this source with no great difficulty. It was found, however, that the overall yield on 3 : 4-epoxybut-1-ene was low (15%) and that the product, which consisted predominantly of the *meso*-form, was less free from stereoisomeric impurity than that prepared by Prévost's method. Recently, the direct oxidation of 3 : 4-epoxybut-1-ene to 1 : 2-3 : 4-diepoxybutane by perbenzoic acid was reported by Everett and Kon (*J.*, 1950, 3131) but they did not state whether their product consisted of the DL- or the *meso*-form.

Except for the work of Przybytek, Griner, and Prévost and the recent investigation by Ross (*J.*, 1950, 2257), the properties of 1 : 2-3 : 4-diepoxybutane have been little investigated.

Przybytek (*Ber.*, 1884, **17**, 1093) demonstrated that addition of aniline gives 1 : 4-dianilinobutane-2 : 3-diol but did not investigate the structures of the other adducts which he obtained. In the present investigation, the structures of the products obtained from 1 : 2-3 : 4-diepoxybutane and piperidine, methanol, phenols, naphthalene-β-thiol, and various other reactive substances have been examined, and most of them are shown by oxidation with periodic acid to be 1 : 4-disubstituted derivatives of butane-2 : 3-diol, only those diols bearing hydroxyl groups on adjacent carbon atoms being susceptible to cleavage by this reagent.

The supposed DL- and *meso*-forms of 1 : 2-3 : 4-diepoxybutane reacted with piperidine in ether giving two 1 : 4-dipiperidinobutane-2 : 3-diols, m. p.s 62° and 106°, both of which were oxidised by periodic acid to piperidinoacetaldehyde. The compound of m. p. 62° was, however, oxidised much more rapidly than that of m. p. 106° (see table).

It is known (Criegee, *Sitzungsber. Ges. Beförder. ges. Naturwiss., Marburg*, 1934, **69**, 25; Price and Knell, *J. Amer. Chem. Soc.*, 1942, **64**, 552) that periodic acid oxidises *cis*-glycols more rapidly than the *trans*-isomers. In the case of 1 : 4-dipiperidinobutane-2 : 3-diol in sulphuric acid, mutual repulsion of the piperidinium ions will bring the two hydroxyl groups close together in the mean position in the D- or the L-form, but will set them apart in the *meso*-form. The DL-form would therefore be expected to behave in a similar manner to a *cis*-glycol and to be oxidised more rapidly by periodic acid than the *meso*-form. The greater rate of oxidation of the 1 : 4-dipiperidinobutane-2 : 3-diol of m. p. 62° therefore affords evidence (see table) that

Oxidation of stereoisomeric 1 : 4-*dipiperidinobutane*-2 : 3-*diols at a concentration of* 12 *g./l. in* 0·05M-*potassium periodate*–0·25N-*sulphuric acid solution.*

Time, mins.		25	90	100	125	1080	1530	2590
% Oxidation of 2 : 3-diol	m. p. 106°	6·5	16	—	—	67	82	100
	m. p. 62°	—	—	100	—	—	—	—

this substance is the DL-form and that the isomeric compound of m. p. 106° is the *meso*-form. As the addition of piperidine to 1 : 2-3 : 4-diepoxybutane does not involve an addition or replacement at an asymmetric carbon atom, it may be concluded that the stereoisomeric compounds from which the 1 : 4-dipiperidinobutane-2 : 3-diols of m. p. 62° and 106° are derived are the DL- and the *meso*-form of 1 : 2-3 : 4-diepoxybutane respectively.

meso-1 : 2-3 : 4-Diepoxybutane reacted with methanol in the presence of a trace of perchloric acid to give 1 : 4-dimethoxybutane-2 : 3-diol, which was oxidised by periodic acid to methoxyacetaldehyde (identified as the 2 : 4-dinitrophenylhydrazone), thus demonstrating its structure.

On treatment with aqueous sodium phenoxide, 1 : 2-3 : 4-diepoxybutane yielded 4-phenoxybutane-1 : 2 : 3-triol (I) and 1 : 4-diphenoxybutane-2 : 3-diol (II). The former compound readily

(I.) $PhO{\cdot}CH_2{\cdot}CH(OH){\cdot}CH(OH){\cdot}CH_2{\cdot}OH$ $\qquad$ $PhO{\cdot}CH_2{\cdot}CH(OH){\cdot}CH(OH){\cdot}CH_2{\cdot}OPh$ (II.)

consumed 2 molecular proportions of periodic acid in dilute sulphuric acid but (II) was not attacked under these conditions, probably on account of its very low solubility in aqueous solutions. In acetic acid containing a small proportion of water, however, oxidation of (II) occurred readily with formation of phenoxyacetaldehyde.

With aqueous sodium *p*-chlorophenoxide, 1 : 2-3 : 4-diepoxybutane gave 4-*p*-chlorophenoxybutane-1 : 2 : 3-triol and 1 : 4-di-*p*-chlorophenoxybutane-2 : 3-diol, but with sodium β-naphthyloxide and sodium β-thionaphthyloxide, only 1 : 4-di-β-naphthyloxy- and 1 : 4-di-β-naphthylthio-butane-2 : 3-diol respectively were identified. The very low solubilities of the latter substances in practically all solvents in the cold precluded demonstration of their structures by periodate oxidation and the above formulations are given only by analogy with the phenoxy-derivatives.

Condensation of *meso*-1 : 2-3 : 4-diepoxybutane with ethyl sodiomalonate and treatment of the product with acid and then with ammonia yielded a substance which has been formulated as 1 : 1′ : 6 : 6′-tetracarbamylhexane-3 : 4-diol (III). This substance was oxidised by periodic acid in dilute sulphuric acid but the product was not identified.

$$(H_2N{\cdot}CO)_2CH{\cdot}CH_2{\cdot}CH(OH){\cdot}CH(OH){\cdot}CH_2{\cdot}CH(CO{\cdot}NH_2)_2 \qquad \text{(III.)}$$

Treatment of 1 : 2-3 : 4-diepoxybutane with ethylmagnesium bromide and subsequent decomposition of the magnesium complex with hydrobromic acid yielded 1 : 4-dibromobutane-2 : 3-diol. Reactions of a similar type have been reported between epichlorohydrin and ethylmagnesium bromide (Koelsch and McElvain, *J. Amer. Chem. Soc.*, 1929, **51**, 3390).

Isomerisation of 1 : 2-3 : 4-diepoxybutane occurred readily on heating it with zinc chloride. The only carbonyl compound identified among the products was diacetyl.

Experimental.

But-2-ene-1 : 4-*diol*.—This was prepared from 1 : 4-dibromobut-2-ene (m. p. 53—54°) either by Prévost's method (*Compt. rend.*, 1926, **183**, 1292) or by direct hydrolysis as follows: 1 : 4-dibromobut-2-ene (138 g.), lead monoxide (286 g.), and water (500 c.c.) were boiled under reflux for 20 hours, the reaction mixture was filtered, and the filtrate concentrated to 100 c.c. The residue was fractionated under reduced pressure, giving but-2-ene-1 : 4-diol (27·2 g., 49%), b. p. 130—140°/16 mm.

meso-1 : 2-3 : 4-*Diepoxybutane*.—But-2-ene-1 : 4-diol was brominated in ether (Prévost, *loc. cit.*), giving 2 : 3-dibromobutane-1 : 4-diol (m. p. 134°), which yielded *meso*-1 : 2-3 : 4-diepoxybutane (m. p. −19°) when treated with potassium hydroxide in ether (Prévost, *loc. cit.*).

DL-1 : 2-3 : 4-*Diepoxybutane*.—1 : 4-Dibromobut-2-ene (m. p. 53—54°) was oxidised to 1 : 4-dibromobutane-2 : 3-diol with dilute potassium permanganate (Griner, *Compt. rend.*, 1893, **117**, 554), and the diol converted into DL-1 : 2-3 : 4-diepoxybutane by potassium hydroxide in ether (*idem*, *ibid.*).

meso-1 : 2-3 : 4-*Diepoxybutane from* 3 : 4-*Epoxybut*-1-*ene*.—3 : 4-Epoxybut-1-ene (308 g.) gave on treatment with hydrochloric acid (Kadesch, *loc. cit.*) 2-chlorobut-3-en-1-ol (300 g., 64%); this was converted into a mixture of butadiene dichlorohydrins (310 g., 44% on epoxybutene), which were separated by Evans and Owen's method (*loc. cit.*). The three fractions obtained, *viz.*, (i) solid 2 : 3-dichlorobutane-1 : 4-diol, (ii) the dichlorobutanediol, b. p. 117—130°/1 mm., and (iii) 2 : 4-dichlorobutane-1 : 3-diol, were separately converted into 1 : 2-3 : 4-diepoxybutane by solid potassium hydroxide in ether. The three products thus obtained were characterised by treatment with piperidine in ether and subsequent examination of the 1 : 4-dipiperidinobutane-2 : 3-diols thus formed.

The product obtained from fraction (i) and potassium hydroxide was homogeneous and consisted of almost pure *meso*-1 : 2-3 : 4-diepoxybutane, the piperidino-compound melting sharply at 106°. Fraction (ii), however, gave with potassium hydroxide a mixture of 1 : 2-3 : 4-diepoxybutane (b. p. 140—142°/761 mm.) and a chlorine-containing substance (b. p. 176—180°/16 mm.) in almost equal quantities. The diepoxybutane was somewhat impure *meso*-form, and gave a piperidino-compound, m. p. 92—100°, raised to 106° by several recrystallisations. Fraction (iii) gave predominantly *meso*-1 : 2-3 : 4-diepoxybutane (piperidino-compound, m. p. 95—102°; 106° after several recrystallisations) in poor yield. Treatment of the mixed dichlorohydrins (310 g.) with potassium hydroxide in ether without previous separation gave *meso*-1 : 2-3 : 4-diepoxybutane (54 g., 14—15% on epoxybutene) satisfactory for use where complete absence of stereoisomeric impurity was not essential.

meso-1 : 4-*Dipiperidinobutane*-2 : 3-*diol*.—*meso*-1 : 2-3 : 4-Diepoxybutane (3 g.) in anhydrous ether (15 c.c.) was mixed with piperidine (6 g.) in ether (20 c.c.). After 48 hours at room temperature, easily volatile matter was removed by gentle warming under reduced pressure. The residue was redissolved in ether, and the solution treated with carbon and filtered. When most of the ether was allowed to evaporate at ordinary temperature, crystals of meso-1 : 4-*dipiperidinobutane*-2 : 3-*diol* (3·6 g.) separated; after recrystallisation from ether, these had m. p. 106° (Found: C, 65·95; H, 10·85; N, 10·95. $C_{14}H_{28}O_2N_2$ requires C, 65·6; H, 10·9; N, 10·9%). The dihydrochloride, prepared by mixing a solution of the base with ethereal hydrogen chloride, had m. p. 298°.

DL-1 : 4-*Dipiperidinobutane*-2 : 3-*diol*.—(*a*) A solution of 1 : 4-dibromobutane-2 : 3-diol (30 g.) (m. p. 85°) in benzene (50 c.c.) was mixed with one of piperidine (42 g.) in benzene (50 c.c.) and refluxed for 1 hour, a gentle reaction taking place with separation of a crystalline product. The slightly sticky product was removed by filtration, the filtrate extracted with 5N-hydrochloric acid (2 × 75 c.c.), and the sticky product dissolved in the hydrochloric acid extract. The clarified (carbon) solution was basified with sodium hydroxide, cooled, and extracted with ether (4 × 100 c.c.). The ethereal solution was dried ($MgSO_4$) and the ether removed, leaving a residue which set solid. By pouring the dry ethereal solution into an excess of saturated ethereal hydrogen chloride and recrystallising the product from absolute ethanol containing some hydrogen chloride, DL-1 : 4-*dipiperidinobutane*-2 : 3-*diol dihydrochloride*, m. p. 258—259°, was obtained (Found: N, 8·0; Cl, 21·1. $C_{14}H_{30}O_2N_2Cl_2$ requires N, 8·5; Cl, 21·6%). Treatment of a solution of the hydrochloride in methanol with 2 equivalents of sodium methoxide, filtration from sodium chloride, evaporation to dryness, and extraction of the residue with boiling benzene gave DL-1 : 4-*dipiperidinobutane*-2 : 3-*diol*, m. p. 62° after crystallisation from ether (Found: C, 66·05; H, 10·9; N, 10·4. $C_{14}H_{28}O_2N_2$ requires C, 65·6; H, 10·9; N, 10·9%).

(*b*) DL-1 : 2-3 : 4-Diepoxybutane (2·7 g.) in anhydrous ether (13·5 c.c.) was mixed with piperidine (5·4 g.) in ether (20 c.c.). After 2 days at room temperature, the ether was removed, and the oily residue converted into the hydrochloride by treatment with ethereal hydrogen chloride. The hydrochloride was purified and the base regenerated as in (*a*), giving a crystalline product, m. p. and mixed m. p. with (*a*), 62°.

Periodic Acid Oxidation of the Stereoisomeric 1 : 4-*Dipiperidinobutane*-2 : 3-*diols*.—The compounds were shaken with a 5—10% excess of 0·05M-potassium periodate in 0·25N-sulphuric acid at 20° until completely dissolved. At intervals, aliquot portions (10 ml.) of the solution were withdrawn and run into 0·1N-sodium arsenite (25 ml.) containing excess of sodium hydrogen carbonate. After addition of potassium iodide solution (1 c.c.; 20% w/v) and 15 minutes' storage the excess of arsenite was determined by titration with 0·1N-iodine solution. When oxidation by periodic acid was complete, the solution containing the oxidation products was treated with sulphur dioxide until the colour of the iodine at first liberated by this reagent had just been discharged. After removal of excess of sulphur dioxide under reduced pressure, the solution was treated with a slight excess of semicarbazide hydrochloride and sufficient sodium acetate to remove the acidity towards Congo-red. The solution was then concentrated to about one-tenth of its original volume. On cooling and basification by cautious addition of 2N-sodium hydroxide, *piperidinoacetaldehyde semicarbazone* separated; it crystallised from water in elongated prisms, m. p. 163° (Found: C, 52·1; H, 8·75; N, 30·3. $C_8H_{16}ON_4$ requires C, 52·2; H, 8·7; N, 30·45%). No depression of m. p. was observed on mixing the semicarbazones obtained from DL- and *meso*-dipiperidinobutanediols together or with a sample prepared from bromoacetal (p. 2486).

Piperidinoacetaldehyde Semicarbazone from Bromoacetal.—Piperidinoacetal was prepared by Stoermer and Burkert's method (*Ber.*, 1894, **27**, 2016), bromoacetal being used in place of chloroacetal. Piperidinoacetal (0·8 g.) was boiled under reflux for 10 minutes with 2N-hydrochloric acid (5 c.c.), the solution cooled, sodium acetate (2 g.) and semicarbazide hydrochloride (0·5 g.) were added, and the mixture was heated on the steam-bath for 1 hour. On filtration and basification of the filtrate with sodium hydroxide, piperidinoacetaldehyde semicarbazone, m. p. 163°, separated. The m. p. of this substance is reported erroneously by Stoermer (*Ber.*, 1898, **31**, 2543) as 76°.

1 : 4-*Dimethoxybutane*-2 : 3-*diol.*—*meso*-1 : 2-3 : 4-Diepoxybutane (5 g.) in methanol (50 c.c.) was treated with 1 drop of 65% perchloric acid and boiled under reflux for 2 hours. After removal of excess of methanol under reduced pressure, the product was distilled, giving a colourless liquid, b. p. 115°/10 mm. On treatment with 3 : 5-dinitrobenzoyl chloride in pyridine–benzene, 1 : 4-*bis*-3 : 5-*dinitrobenzoyloxydimethoxybutane* was obtained; it crystallised from 2-ethoxyethanol in pale yellow needles, m. p. 225° (Found: C, 44·7; H, 3·55; N, 10·65. $C_{20}H_{18}O_{14}N_4$ requires C, 44·6; H, 3·35; N, 10·4%).

Oxidation of 1 : 4-*Dimethoxybutane*-2 : 3-*diol with Periodic Acid.*—This oxidation was carried out in the manner described for the dipiperidino-compounds. After reduction of the excess of periodate and iodate with sulphur dioxide and removal of excess of the latter, the solution was shaken with a saturated solution of 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid, and the yellow precipitate collected, washed with water, and crystallised from methanol, giving orange needles, m. p. and mixed m. p. (with authentic sample of methoxyacetaldehyde 2 : 4-dinitrophenylhydrazone) 119° (Found: C, 42·4; H, 4·15; N, 22·55; OCH_3, 11·7. Calc. for $C_9H_{10}O_5N_4$: C, 42·5; H, 3·95; N, 22·05; OCH_3, 12·2%).

1 : 4-*Diphenoxybutane*-2 : 3-*diol.*—(*a*) Phenol (3·8 g.) in water (20 c.c.) and 11N-sodium hydroxide (3·6 c.c.) was treated with *meso*-1 : 2-3 : 4-diepoxybutane (1·8 g.) at 15—20°. The temperature was raised gradually to 100° and kept thereat for 1 hour. After cooling, the crystalline product was filtered off, and washed with 2N-sodium hydroxide and then with water. Crystallisation from ethanol yielded 1 : 4-*diphenoxybutane*-2 : 3-*diol*, m. p. 185° (Found: C, 70·25; H, 6·4. $C_{16}H_{18}O_4$ requires C, 70·05; H, 6·55%). The alkaline liquors were clarified (carbon) and acidified. The crystalline product which separated on storage was filtered off and extracted twice with ether to remove phenol. The residue crystallised from ethanol in the form of white platelets, m. p. 132—133°, of 4-*phenoxybutane*-1 : 2 : 3-*triol* (Found: C, 60·5; H, 6·95. $C_{10}H_{14}O_5$ requires C, 60·6; H, 7·05%). This compound was oxidised by potassium periodate–sulphuric acid solution in the manner previously described, titration showing that 98 g. of compound were oxidised by 1 g.-mol. of periodic acid (HIO_4), *i.e.*, approx. 2 mols. of periodic acid per mol. of triol.

1 : 4-Diphenoxybutane-2 : 3-diol was not oxidised by periodic acid under the usual conditions, as it was very sparingly soluble in water. Its oxidation was effected by rapidly cooling a solution of 0·17 g. in acetic acid (30 c.c.) to 25° and adding sodium periodate (0·17 g.) in dilute sulphuric acid (1 c.c.; 1 : 3). After 20 hours at 25°, water (25 c.c.) was added, the solution treated with sulphur dioxide, excess of the latter removed, and the solution added to a saturated solution of 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid. The product, collected and purified by crystallisation from ethanol, had m. p. 134° (Hatch and Nesbitt, *J. Amer. Chem. Soc.*, 1945, **67**, 39, give m. p. 138° for phenoxyacetaldehyde 2 : 4-dinitrophenylhydrazone).

(*b*) 1 : 4-Diphenoxybutane-2 : 3-diol was obtained in poor yield by heating *meso*-1 : 2-3 : 4-diepoxybutane (0·9 g.) with phenol (1·9 g.) for 10 hours at 135—140° in a sealed tube.

1 : 4-*Di-p-chlorophenoxybutane*-2 : 3-*diol.*—*meso*-1 : 2-3 : 4-Diepoxybutane (1·8 g.) was added to *p*-chlorophenol (5·2 g.) in water (20 c.c.) and 11N-sodium hydroxide (3·6 c.c.), and the temperature raised to 100° during 15 minutes and kept at 100° for 1 hour. 1 : 4-*Di-p-chlorophenoxybutane*-2 : 3-*diol* separated from the liquors and was purified by crystallisation from ethanol; it formed silvery leaflets, m. p. 186—187° (Found: Cl, 20·9. $C_{16}H_{14}O_4Cl_2$ requires Cl, 20·7%). The liquors gave on acidification 4-*p-chlorophenoxybutane*-1 : 2 : 3-*triol*, silvery plates (from ethanol), m. p. 136° (Found: C, 51·6; H, 5·7; Cl, 15·2. $C_{10}H_{13}O_4Cl$ requires C, 51·6; H, 5·6; Cl, 15·25%).

1 : 4-*Di-β-naphthyloxybutane*-2 : 3-*diol* was obtained in a similar manner to the corresponding phenoxy-derivative, β-naphthol (5·8 g.) being used in place of phenol. It formed silvery white leaflets (from 2-ethoxyethanol), m. p. 253—254° (Found: C, 77·0; H, 5·4. $C_{24}H_{22}O_4$ requires C, 77·0; H, 5·9%).

1 : 4-*Di-β-naphthylthiobutane*-2 : 3-*diol.*—Naphthalene-β-thiol (3·2 g.) in water (20 c.c.) and 10N-sodium hydroxide (2·5 c.c.) was treated with *meso*-1 : 2-3 : 4-diepoxybutane (0·9 g.) at 50°. After being heated on the steam-bath for 1 hour, the reaction mixture was cooled, and the product filtered off, and washed with 2N-sodium hydroxide, water, and then methanol. Crystallised from ethanol and then from 2-ethoxyethanol the *diol* formed rosettes of needles, m. p. 167° (Found: C, 70·6; H, 5·6; S, 16·0. $C_{24}H_{22}O_2S_2$ requires C, 70·95; H, 5·4; S, 15·75%).

Condensation of 1 : 2-3 : 4-*Diepoxybutane with Ethyl Sodiomalonate.*—Sodium (2·3 g.) in ethanol (40 c.c.) was treated with ethyl malonate (16 g.), and *meso*-1 : 2-3 : 4-diepoxybutane (4·3 g.) added. The mixture became warm and began to deposit a yellow precipitate. After 30 minutes at room temperature, it was boiled under reflux for 20 minutes and cooled, the precipitate filtered off, and the ethanol removed from the filtrate. The residue and precipitate were united and treated with an excess of dilute sulphuric acid (1 : 6), giving an oil which was extracted with ether (3 × 40 c.c.). The extract was dried ($MgSO_4$), and the ether and excess of ethyl malonate were removed from the product by distillation. The residue was dissolved in ethanol and warmed with excess of aqueous ammonia (*d* 0·88). A crystalline precipitate of 1 : 1 : 6 : 6-*tetracarbamylhexane*-3 : 4-*diol* separated; purified by crystallisation from ethanol (carbon), it had m. p. 225—227° (decomp.) (Found: C, 41·3; H, 6·65; N, 19·1. $C_{10}H_{16}O_6N_4$ requires C, 41·4; H, 6·2; N, 19·3%).

On treatment with potassium periodate in water and subsequent removal of excess of periodate with sulphur dioxide, the tetra-amide was oxidised to a substance giving a yellow precipitate with 2 : 4-dinitrophenylhydrazine, but owing to the very small amount of material this was not identified.

Condensation of 1 : 2-3 : 4-*Diepoxybutane with Ethylmagnesium Bromide.*—*meso*-1 : 2-3 : 4-Diepoxybutane (4·3 g.) in ether (25 c.c.) was added dropwise to ethylmagnesium bromide prepared from magnesium (2·5 g.) and ethyl bromide (11·5 g.) in ether (60 c.c.), the mixture being cooled in an ice-bath. After 2 hours at room temperature, the reaction mixture was refluxed for 1½ hours and set aside for 65 hours. Dilute hydrobromic acid was then added until the initial precipitate had just dissolved, after which the ethereal layer was separated, and the aqueous layer extracted with more ether (3 × 100 c.c.). The ethereal extract was dried ($MgSO_4$ + $NaHCO_3$), the ether removed, and the residue distilled under reduced pressure (b. p. 134°/14 mm.), giving silvery plates, m. p. 107—111°, raised to 134—135° by crystallisation from benzene (Found: C, 19·65; H, 3·15. Calc. for $C_4H_8O_2Br_2$: C, 19·35; H, 3·25%).

Isomerisation of 1 : 2-3 : 4-*Diepoxybutane.*—1 : 2-3 : 4-Diepoxybutane (2·2 g.) was mixed with fused zinc chloride (0·5 g.), the mixture becoming hot spontaneously. After 2 hours, it was distilled cautiously, giving a distillate (1·6 g.) which was redistilled. The product (0·4 g.), b. p. 85—95°, gave a phenylhydrazone, m. p. 250° not depressed on admixture with an authentic sample of diacetyl bisphenylhydrazone (Found: C, 72·25; H, 6·7; N, 21·6. Calc. for $C_{16}H_{18}N_4$: C, 72·2; H, 6·75; N, 21·1%).

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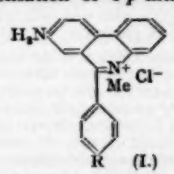
548. *Syntheses of Some Derivatives of 2-Phenyl-1-azapyrene.*

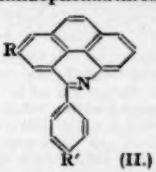
By J. W. COOK and J. S. MOFFATT.

Syntheses of quaternary ammonium salts of 2-phenyl-, 2-*p*-aminophenyl-, 4-carbethoxyamino-2-*p*-nitrophenyl-, and 2-*p*-aminophenyl-4-carbethoxyamino-1-azapyrene are described. Some of these compounds show activity as trypanocides but are inferior, in this respect, to analogous phenanthridinium compounds.

WALLS and others (see, *e.g.*, *J. Soc. Chem. Ind.*, 1947, **66**, 182) have shown that marked trypanocidal activity is associated with certain quaternary ammonium salts of 9-phenylphenanthridine containing suitably located substituents such as amino, nitro or methoxyl. An important condition for the development of activity is the presence of at least one such substituent in the phenanthridine ring. Phenidium chloride (I; R = NH_2), which is one of the most active members of the series against *Trypanosoma congolense*, retains activity even if one of its amino-groups is replaced by nitro, to give (I; R = NO_2) (Walls, Browning, Calver, and Leckie, *J.*, 1947, 67). The object of the present work was to prepare similarly substituted derivatives of the analogous ring-system, 2-phenyl-1-azapyrene (II; R = R′ = H) (Cook and Thomson, *J.*, 1945, 395). This polycyclic base readily yielded a quaternary methobromide. Cyclisation of 4-*p*-nitrobenzamidophenanthrene by means of phosphoryl chloride in nitro-



(I.)



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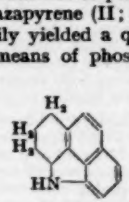

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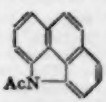

(IV.)

benzene afforded 2-*p*-nitrophenyl-1-azapyrene (II; R = H; R′ = NO_2) which was quaternised and then reduced, etc., to give the methotoluene-*p*-sulphonate of (II; R = H; R′ = NH_2). This amino-quaternary salt had a definite curative action on *T. congolense* infections in mice when administered in doses approaching the maximum tolerated. For the preparation of derivatives of (II) containing an amino-, or substituted amino-, group in the azapyrene part of the molecule, it was considered that the required intermediate 4-amino-*x*-nitrophenanthrene (where *x* is any position other than 5) might be prepared by nitration of 1 : 2 : 3 : 4-tetrahydro-4-ketophenanthrene, followed by application of the Schroeter reaction (*Ber.*, 1930, **63**, 1308) to the oxime of the resulting nitro-ketone. However, under the various conditions tried, a homogeneous mononitro-derivative was readily obtained only by nitration in presence of acetic anhydride. The product, obtained in about 50% yield, must be 1 : 2 : 3 : 4-tetrahydro-4-keto-5-nitrophenanthrene because (*a*) it gave no oxime and (*b*) it gave, on catalytic hydrogenation, 1 : 2 : 3 : 4-tetrahydro-4 : 5-iminophenanthrene (III), which, on acetylation followed by

dehydrogenation, yielded *N*-acetyl-4 : 5-iminophenanthrene (IV). Nitration of 1 : 2 : 3 : 4-tetrahydro-1-ketophenanthrene under similar conditions was then studied in the hope that the nitro-group might likewise enter the 5-position. The product, however, consisted of a 1 : 2 : 3 : 4-tetrahydro-1-keto-*x*-nitrophenanthrene whose structure was determined as follows. Treatment of its oxime with hydrogen chloride in a mixture of acetic acid and acetic anhydride according to Schroeter's method (*loc. cit.*) afforded a 1-amino-nitrophenanthrene which, on diazotisation followed by hypophosphorous acid reduction of its diazonium salt, yielded 9-nitrophenanthrene. If the normal directive effect of a carbonyl group attached to an aromatic system is borne in mind, it is probable that the nitration product is 1 : 2 : 3 : 4-tetrahydro-1-keto-9-nitrophenanthrene, but the possibility of its being the 10-nitro-isomer is not rigidly excluded. The required intermediate was eventually obtained by carbethoxylation of 7-amino-1 : 2 : 3 : 4-tetrahydro-4-ketophenanthrene (Miyasaka, *Chem. Abstr.*, 1940, **34**, 7288), followed by Schroeter treatment of the carbethoxyamino-ketone oxime, which yielded 5-amino-2-carbethoxyaminophenanthrene. Similar treatment of the oxime of 1 : 2 : 3 : 4-tetrahydro-4-keto-7-methoxyphenanthrene yielded 5-amino-2-methoxyphenanthrene. These reactions, involving the conversion of 1 : 2 : 3 : 4-tetrahydro-4-ketophenanthrene oximes into 4-phenanthrylamines, gave yields of the order of 20% and neutral by-products were frequently encountered.

Cyclisation of 4-benzamidophenanthrenes by means of phosphoric oxide in xylene (Cook and Thomson, *loc. cit.*) proceeded smoothly only in the case of the unsubstituted derivative. In other cases, phosphoryl chloride alone, or preferably in nitrobenzene solution, gave rather better results although it was not invariably successful. For example, 4-*p*-carbethoxyaminobenzamidophenanthrene suffered hydrolysis of the ester grouping as well as cyclisation, a result which is in contrast to the smooth cyclisation of 2-*p*-carbethoxyaminobenzamidodiphenyl derivatives previously reported by Walls (*loc. cit.*; *J.*, 1948, 188). Cyclisation of 2-carbethoxyamino-5-*p*-nitrobenzamidophenanthrene followed by quaternisation yielded the methosulphate of 4-carbethoxyamino-2-*p*-nitrophenyl-1-azapyrene (II; R = $NH{\cdot}CO_2Et$, R′ = NO_2), which, on reduction with iron powder, yielded the methosulphate of 2-*p*-aminophenyl-4-carbethoxyamino-1-azapyrene (II; R = $NH{\cdot}CO_2Et$, R′ = NH_2). The nitro- and amino-quaternary salts showed definite curative actions on *T. congolense* infections in mice but did not show the range of activity possessed by the best of the phenanthridinium compounds. For this reason, and also because of lack of material, attempts were not made to hydrolyse (II; R = $NH{\cdot}CO_2Et$, R′ = NH_2) to a diamine which would be exactly analogous to phenidium chloride.

Experimental.

For the preparation of 1 : 2 : 3 : 4-tetrahydro-4-ketophenanthrene, γ-(β-naphthyl)butyric acid was prepared (on the 100-g. scale) by palladium–charcoal dehydrogenation of methyl γ-(1 : 2 : 3 : 4-tetrahydro-6-naphthyl)butyrate (Newman and Zahm, *J. Amer. Chem. Soc.*, 1943, **65**, 1097). The procedure, recommended by these authors, of dehydrogenation of methyl β-(1 : 2 : 3 : 4-tetrahydro-2-naphthoyl)-propionate, although, in our hands, satisfactory on a small scale (5 g.), was not when applied to larger batches (100 g.) of material. The product then consisted of a mixture containing much β-(β-naphthoyl)-propionic acid and also some not fully aromatised material. γ-(β-Naphthyl)butyric acid was cyclised by means of anhydrous hydrogen fluoride to give 1 : 2 : 3 : 4-tetrahydro-4-ketophenanthrene in 80% yield.

Nitration of 1 : 2 : 3 : 4-*Tetrahydro*-4-*ketophenanthrene*.—A mechanically stirred solution of the ketone (5 g.) in glacial acetic acid (6·4 c.c.) was cooled at 0° and treated dropwise, during 15 minutes, with a cold mixture of acetic anhydride (3·8 c.c.) and nitric acid (98%; 2·5 c.c.). The mixture was kept at 0° for 4 hours. The crystalline product was filtered off, washed with a small quantity of methanol, and then recrystallised from ethanol, giving rhombs (3·0 g., 49%), m. p. 162°, of 1 : 2 : 3 : 4-*tetrahydro*-4-*keto*-5-*nitrophenanthrene* (Found: C, 69·8; H, 4·6; N, 6·0. $C_{14}H_{11}O_3N$ requires C, 69·7; H, 4·6; N, 5·8%). It was recovered unchanged after being boiled under reflux with hydroxylamine hydrochloride in anhydrous pyridine for several hours.

Oxidation of 1 : 2 : 3 : 4-*Tetrahydro*-4-*keto*-5-*nitrophenanthrene*.—The finely powdered nitro-ketone (0·3 g.) was boiled under reflux with nitric acid (*d* 1·42; 2 c.c.) and water (24 c.c.) for 90 hours. The mixture was cooled and the yellow solid filtered off. It was extracted with warm, 2N-sodium carbonate (5 c.c.). The extract, on acidification with concentrated hydrochloric acid, very slowly deposited crystals which, on recrystallisation from water, formed large, yellow prisms, m. p. 232—234°, of 8-*nitronaphthalene*-1 : 2-*dicarboxylic acid* (Found: C, 54·8; H, 2·9. $C_{12}H_7O_6N$ requires C, 55·1; H, 2·7%).

Catalytic Hydrogenation of 1 : 2 : 3 : 4-*Tetrahydro*-4-*keto*-5-*nitrophenanthrene*.—A suspension of the nitro-ketone (0·5 g.) in absolute ethanol (40 c.c.) was shaken with hydrogen in presence of a 20% palladium–charcoal catalyst (0·2 g.; Newman and Zahm, *loc. cit.*). The mixture rapidly absorbed 3 mols. of hydrogen; hydrogenation was stopped when a further 1·1 mols. had been slowly taken up. The catalyst was filtered off and the filtrate evaporated under reduced pressure. The residual gum, on trituration with ethanol, gave a solid which, on recrystallisation from ethanol, formed white needles (0·1 g.), m. p. 113°, of 1 : 2 : 3 : 4-*tetrahydro*-4 : 5-*iminophenanthrene* (III) (Found: C, 85·8; H, 6·8; N, 6·9. $C_{14}H_{13}N$ requires C, 86·2; H, 6·7; N, 7·2%). Crystals of this substance rapidly darkened on exposure to the atmosphere, and in subsequent experiments it was found preferable to acetylate the

crude hydrogenation product by heating it with acetic anhydride (5 c.c.) on a water-bath for 3 hours. After removal of acetic anhydride under reduced pressure, the residue was recrystallised (charcoal) from methanol, giving prisms (0·26 g.), m. p. 136°, of *N-acetyl*-1 : 2 : 3 : 4-*tetrahydro*-4 : 5-*iminophenanthrene* (Found : C, 80·9; H, 6·1; N, 5·9. $C_{16}H_{15}ON$ requires C, 81·0; H, 6·4; N, 5·9%). Dehydrogenation of this acetyl derivative by heating the substance (100 mg.) with 20% palladium–charcoal (50 mg.) at 180—250° in an atmosphere of carbon dioxide for 1 hour, yielded *N-acetyl*-4 : 5-*iminophenanthrene* (IV), which formed long, white needles (from ethanol), m. p. 174—175° (Found : C, 82·7; H, 5·0; N, 6·0. $C_{16}H_{11}ON$ requires C, 82·4; H, 4·8; N, 6·0%).

Nitration of 1 : 2 : 3 : 4-*Tetrahydro*-1-*ketophenanthrene*.—A solution of the ketone (10 g.) in acetic acid (14 c.c.)–acetic anhydride (4 c.c.) was mechanically stirred and rapidly cooled to 0°. The resulting suspension was treated dropwise, during 30 minutes, with a cold mixture of nitric acid (98%; 5 c.c.) and acetic anhydride (4 c.c.). The mixture was kept at 0° for 3 hours longer. The crystalline product was collected, washed with a small quantity of aqueous ethanol, and then recrystallised from ethanol, giving yellow needles (3·7 g.), m. p. 147—148°, of 1 : 2 : 3 : 4-*tetrahydro*-1-*keto*-9-*nitrophenanthrene* (Found : C, 70·1; H, 4·6; N, 5·8. $C_{14}H_{11}O_3N$ requires C, 69·7; H, 4·6; N, 5·8%).

Catalytic Hydrogenation of 1 : 2 : 3 : 4-*Tetrahydro*-1-*keto*-9-*nitrophenanthrene*.—A suspension of the nitro-ketone (300 mg.) in absolute ethanol (25 c.c.) was hydrogenated in presence of 10% palladium–charcoal (150 mg.). Uptake ceased when 3 mols. of hydrogen had been absorbed. The product consisted of a mixture of a high-melting, neutral solid and a basic fraction which, on treatment with hot 2N-hydrochloric acid, yielded tan-coloured prisms, m. p. 229—231°, of 9-*amino*-1 : 2 : 3 : 4-*tetrahydro*-1-*ketophenanthrene hydrochloride* (Found : C, 67·6; H, 5·7; N, 5·9. $C_{14}H_{13}ON$,HCl requires C, 67·8; H, 5·7; N, 5·7%).

1 : 2 : 3 : 4-*Tetrahydro*-1-*keto*-9-*nitrophenanthrene Oxime*.—The ketone (3·1 g.) was heated under reflux on a water-bath with hydroxylamine hydrochloride (1·9 g.), ethanol (50 c.c.), and pyridine (20 c.c.) for 2·5 hours. The solvents were removed under reduced pressure. The residue was triturated with water, to give a solid (3·2 g.; m. p. 194—196°) which, on recrystallisation from ethanol, formed yellow rhombs, m. p. 202—203° (Found : N, 10·8. $C_{14}H_{12}O_3N_2$ requires N, 10·9%).

1-*Amino*-9-*nitrophenanthrene*.—A suspension of the foregoing oxime (2·25 g.) in acetic acid (34 c.c.)–acetic anhydride (4·5 c.c.) was heated on a water-bath and treated with a stream of anhydrous hydrogen chloride during 8 hours. The resulting solution was cooled and set aside at room temperature for 3 days. The crystalline material (1·9 g.) which separated was collected and extracted with boiling 2N-hydrochloric acid (1500 c.c.). The insoluble residue (0·5 g.) was extracted with boiling benzene (150 c.c.). The extract was cooled and then percolated through a short column of activated alumina. The column was washed with benzene. Evaporation of the benzene filtrates furnished a crystalline solid (0·2 g.) which, on recrystallisation from ethanol, formed pale buff-coloured needles, m. p. 208—210°, of a *substance* (Found : C, 63·1; H, 4·7; N, 5·6. $C_{13}H_{11}O_4N$ requires C, 63·6; H, 4·5; N, 5·7%). Basification, with concentrated ammonia solution, of the hydrochloric acid extract gave a precipitate (1 g.) which was collected, washed with water, and purified by chromatography from benzene solution on alumina. The adsorbed base was eluted by means of 0·2% methanol–benzene. It formed crimson needles (0·7 g.) (from ethanol), m. p. 171—173°, of 1-*amino*-9-*nitrophenanthrene* (Found : C, 70·2; H, 4·1; N, 11·9. $C_{14}H_{10}O_2N_2$ requires C, 70·6; H, 4·2; N, 11·8%).

Deamination of 1-*Amino*-9-*nitrophenanthrene*.—A solution of nitrosylsulphuric acid, prepared by portion-wise addition of sodium nitrite (60 mg.) to a cold mixture of water (0·29 c.c.) and concentrated sulphuric acid (*d* 1·84; 0·58 c.c.) followed by warming on a water-bath, was cooled to 0° and treated dropwise, with stirring, with a solution of the nitro-amine (100 mg.) in pyridine (0·5 c.c.). The mixture was cooled at 0° for a further 30 minutes, and then treated with ice-cold hypophosphorous acid solution (30%; 3 c.c.). The mixture was maintained at 0° for 24 hours, diluted with water, and then filtered. The residue was dissolved in benzene (50 c.c.). The solution was washed with dilute sodium hydroxide solution, then with water, and concentrated to small bulk. The solution was poured on a column of activated alumina. The column was washed with benzene until the lower, faintly-yellow band had passed into the filtrate. Evaporation of the latter gave a crystalline residue which, on recrystallisation from ethanol, formed yellow needles (49 mg.), m. p. 113—114°, of 9-nitrophenanthrene (Found : C, 75·0; H, 4·0; N, 6·6. Calc. for $C_{14}H_9O_2N$: C, 75·3; H, 4·1; N, 6·3%). (Keyes and Brooker, *J. Amer. Chem. Soc.*, 1937, **59**, 74, give m. p. 112·5—114·5°.) Hydrogenation, in presence of 20% palladium–charcoal (10 mg.), of the nitro-derivative (28 mg.), dissolved in ethanol (10 c.c.), yielded a basic product (20 mg.) of indefinite m. p. (shrinks at 104°, liquefies at 113—120°). According to Schmidt and Heinle (*Ber.*, 1911, **44**, 1488) 9-aminophenanthrene exists in two forms, one of m. p. 137—138° and the other of m. p. 104°. Acetylation of the crude base in benzene, with acetic anhydride, yielded a homogeneous acetyl derivative which formed glistening needles (from methanol), m. p. 210—211° (Keyes and Brooker, *loc. cit.*, give m. p. 213—215° for 9-acetamidophenanthrene).

γ-(6-*Methoxy*-2-*naphthyl*)*butyric Acid*.—Reduction of β-(6-methoxy-2-naphthoyl)propionic acid (Short, Stromberg, and Wiles, *J.*, 1936, 319) gave more favourable yields by the Huang-Minlon modification of the Wolff–Kishner method (*J. Amer. Chem. Soc.*, 1946, **68**, 2487) than by the Clemmensen method (cf. Kupchan and Elderfield, *J. Org. Chem.*, 1946, **11**, 136). A mixture of the keto-methoxy-acid (51·6 g.), potassium hydroxide (37·6 g.), diethylene glycol (260 c.c.), and hydrazine hydrate (90%; 52 c.c.) was boiled under reflux for 1 hour. It was then slowly distilled until the temperature of the mixture reached 190°. The residue was boiled under reflux for 3·5 hours, cooled, diluted with water (260 c.c.), and then poured into N-hydrochloric acid (1500 c.c.). The precipitate consisted largely of γ-(6-*hydroxy*-2-*naphthyl*)*butyric acid*. A small sample, distilled at 200—210°/0·3 mm., followed by repeated recrystallisation from acetone, formed clusters of rhombs, m. p. 150—152° (Found : C, 73·4; H, 6·1. $C_{14}H_{14}O_3$ requires C, 73·0; H, 6·1%). The main bulk was dissolved in a solution of sodium hydroxide (48 g.) in water (600 c.c.). The solution was heated to 60° and then treated dropwise, with

stirring, with methyl sulphate (50 g.). The mixture was heated to 80° for a further hour, cooled, diluted with water (700 c.c.), and then set aside for 12 hours. The sparingly soluble sodium salt which separated was filtered off. Acidification of the filtrate with concentrated hydrochloric acid gave a precipitate (29 g.) which was distilled at 206—215°/0·2 mm. The distillate was recrystallised from acetone to give crystals (20·5 g.), m. p. 129—132°, of γ-(6-methoxy-2-naphthyl)butyric acid. A further quantity (12·5 g.) of pure material, m. p. 134—136°, was obtained by acidification of the above-mentioned sodium salt.

1 : 2 : 3 : 4-*Tetrahydro*-4-*keto*-7-*methoxyphenanthrene* (cf. Miyasaka, *Chem. Abstr.*, 1940, **34**, 1012).—A solution of γ-(6-methoxy-2-naphthyl)butyric acid (10 g.) in anhydrous benzene (150 c.c.) was treated portionwise with phosphoric oxide (50 g.). The mixture was boiled under reflux on a water-bath for 3 hours. It was cooled and then decomposed with crushed ice (250 g.). Sodium chloride (30 g.) and ether (150 c.c.) were added. The organic layer was separated, washed with brine, filtered, and evaporated. The residual gum was dissolved in ether (100 c.c.). The crystalline solid which separated slowly, was filtered off and recrystallised from benzene, to give clusters of needles (0·8 g.), m. p. 130—132°, of γ-(6-*methoxy*-2-*naphthyl*)*butyric anhydride* (Found: C, 76·4; H, 6·5; OMe, 13·2. $C_{28}H_{28}O_5$ requires C, 76·6; H, 6·4; OMe, 13·2%). The residue obtained by evaporation of the filtrate distilled as a pale-yellow oil, b. p. 172—175°/0·3 mm., which crystallised on cooling to give prisms (6·0 g.), m. p. 52°, of 1 : 2 : 3 : 4-tetrahydro-4-keto-7-methoxyphenanthrene (Found: C, 79·3; H, 6·2. Calc. for $C_{15}H_{14}O_2$: C, 79·6; H, 6·2%). This was also obtained (yield, 81%) by anhydrous hydrogen fluoride treatment of γ-(6-methoxy-2-naphthyl)butyric acid. With hydroxylamine hydrochloride in boiling pyridine it gave an *oxime* which separated from ethanol in rhombs, m. p. 174° (Found: N, 5·8. $C_{15}H_{15}O_2N$ requires N, 5·8%).

1 : 2 : 3 : 4-*Tetrahydro*-7-*hydroxy*-4-*ketophenanthrene*.—A solution of the foregoing methoxy-ketone (15·5 g.) in anhydrous benzene (160 c.c.) was boiled under reflux with powdered, anhydrous aluminium chloride (24 g.) for 4 hours. The mixture was cooled and then poured into a mixture of crushed ice and hydrochloric acid. The precipitate was filtered off, washed with dilute hydrochloric acid and water, and then dissolved in 2N-sodium hydroxide. The solution was clarified by extraction with a small quantity of ether and then acidified with concentrated hydrochloric acid. The precipitated hydroxy-ketone (13·6 g.; m. p. 182—186°) was sufficiently pure for the next stage. A sample crystallised from methanol in elongated prisms, m. p. 186—187° (Found: C, 79·4; H, 5·7. Calc. for $C_{14}H_{12}O_2$: C, 79·2; H, 5·7%) (Miyasaka, *loc. cit.*, is quoted to give m. p. 117°). The hydroxy-ketone was also obtained directly by cyclisation of γ-(6-hydroxy-2-naphthyl)butyric acid with anhydrous hydrogen fluoride.

7-*Amino*-1 : 2 : 3 : 4-*tetrahydro*-4-*ketophenanthrene* (cf. Miyasaka, *loc. cit.*, p. 7288).—A mixture of the aforesaid hydroxy-ketone (2 g.), dioxan (4 c.c.), and saturated sodium hydrogen sulphite solution (6 c.c.) was treated dropwise with concentrated ammonia solution (*d* 0·88; 8 c.c.) and then heated for 19 hours at 180—190° in a sealed tube. The contents of four such tubes were poured into water. The precipitate was filtered off, washed with water, and dissolved in chloroform (200 c.c.). The solution was extracted twice with 2N-sodium hydroxide. Acidification of the extracts gave 1·3 g. of somewhat tarry starting material. The chloroform solution was washed with water and then evaporated. The residue was extracted with boiling N-hydrochloric acid (1500 c.c.). Basification of the extract with concentrated ammonia solution gave a yellow precipitate (3·7 g.) of practically pure amino-ketone, m. p. 153—155°.

7-*Carbethoxyamino*-1 : 2 : 3 : 4-*tetrahydro*-4-*ketophenanthrene*.—A solution of 7-amino-1 : 2 : 3 : 4-tetrahydro-4-ketophenanthrene (3·94 g.) in a mixture of ethanol (80 c.c.) and diethylaniline (3·3 c.c.) was treated dropwise with ethyl chloroformate (1·96 c.c.), boiled under reflux for 30 minutes, and then concentrated on a water-bath to small bulk. The practically pure product (4·56 g.) crystallised on cooling. A further quantity (0·3 g.) was obtained by evaporation of the filtrate and treatment of the residue with dilute hydrochloric acid, followed by recrystallisation of the residue from ethanol. The *carbethoxyamino-ketone* formed clusters of stout needles (from ethanol), m. p. 182—183° (Found: C, 72·3; H, 6·1; N, 4·9. $C_{17}H_{17}O_3N$ requires C, 72·1; H, 6·1; N, 5·0%). With hydroxylamine hydrochloride in boiling pyridine it yielded an *oxime* which formed colourless rhombs (from ethanol), m. p. 167° (Found: N, 9·5. $C_{17}H_{18}O_3N_2$ requires N, 9·4%).

5-*Amino*-2-*carbethoxyaminophenanthrene*.—A solution of 7-carbethoxyamino-1 : 2 : 3 : 4-tetrahydro-4-ketophenanthrene oxime (6·16 g.) in hot glacial acetic acid (37 c.c.) was treated with acetic anhydride (6 c.c.). It was then heated in an oil-bath at 95—100° and treated with a slow stream of anhydrous hydrogen chloride, passed over the surface of the liquid, during 6 hours. The dark mixture was set aside for 3 days and then filtered. The residue was extracted with boiling 0·5N-hydrochloric acid (1200 c.c.). The residue (1·23 g.; m. p. 153—156°), on repeated recrystallisation (charcoal) from ethanol, formed clumps of white needles, m. p. 166°, of a *substance* which has not been identified (Found: C, 70·6; H, 6·0; N, 8·4. $C_{19}H_{18}O_3N_2$ requires C, 70·8; H, 5·6; N, 8·7%). The hydrochloric acid extract, on basification with concentrated ammonia solution, gave a precipitate which was purified by means of chromatography of a benzene solution on alumina followed by elution of the adsorbed base with 0·1% methanol–benzene. The purified *amine* (1·09 g., 19%) formed tan-coloured prisms (from ethanol), m. p. 164° (Found: N, 9·6. $C_{17}H_{16}O_2N_2$ requires N, 9·9%). Addition of acetic anhydride (0·1 c.c.) to a solution of the amine (50 mg.) in boiling ethanol (2 c.c.) yielded the *acetyl* derivative which formed minute, colourless rhombs (from ethanol), m. p. 241° with previous shrinking (Found: C, 70·6; H, 5·8; N, 9·0. $C_{19}H_{18}O_3N_2$ requires C, 70·8; H, 5·6; N, 8·7%).

5-*Amino*-2-*methoxyphenanthrene*.—Treatment of 1 : 2 : 3 : 4-tetrahydro-4-keto-7-methoxyphenanthrene oxime (3·7 g.) in acetic acid (19 c.c.)–acetic anhydride (3·7 c.c.) with anhydrous hydrogen chloride at 95—100° for 6 hours, followed by isolation as described above for 5-amino-2-carbethoxyaminophenanthrene, furnished 5-*amino*-2-*methoxyphenanthrene* which formed red rhombs (yield, 13%) (from ethanol), m. p. 106° (Found: C, 80·3; H, 6·0; N, 6·1. $C_{15}H_{13}ON$ requires C, 80·7; H, 5·8; N, 6·3%).

Derivatives of 4-Benzamidophenanthrene.—These were prepared by portionwise addition of a slight excess of the substituted benzoyl chloride to a solution, cooled at 0°, of the appropriate 4-aminophenanthrene in anhydrous pyridine. After being set aside at room temperature for 24 hours, the pyridine solutions were poured into water and the precipitated amides purified by recrystallisation. 4-p-*Nitrobenzamidophenanthrene* formed pale-yellow needles (from ethanol), m. p. 234° (Found: C, 73·9; H, 4·5; N, 7·9. $C_{21}H_{14}O_3N_2$ requires C, 73·7; H, 4·1; N, 8·2%), 4-p-*carbethoxyaminobenzamidophenanthrene*, needles (from ethanol), m. p. 222° (Found: N, 7·5. $C_{24}H_{20}O_3N_2$ requires N, 7·3%), 2-*methoxy*-5-p-*nitrobenzamidophenanthrene*, pale yellow needles (from acetic acid), m. p. 231—232° (Found: C, 70·8; H, 4·4; N, 7·2. $C_{22}H_{16}O_4N_2$ requires C, 70·9; H, 4·3; N, 7·5%), 2-*carbethoxyamino*-5-p-*nitrobenzamidophenanthrene*, pale-yellow needles (from acetic acid), m. p. 247° (Found: C, 66·7; H, 4·6; N, 9·8. $C_{24}H_{19}O_5N_3$ requires C, 67·1; H, 4·5; N, 9·8%), and 2-*carbethoxyamino*-5-p-*carbethoxyaminobenzamidophenanthrene*, minute rhombs (from acetic acid), m. p. 222° (Found: C, 68·5; H, 5·4; N, 9·0. $C_{27}H_{25}O_5N_3$ requires C, 68·8; H, 5·3; N, 8·9%).

Cyclisation Experiments.

2-*Phenyl*-1-*azapyrene Methobromide.*—Treatment of 4-benzamidophenanthrene with phosphoric oxide in boiling xylene (Cook and Thomson, *loc. cit.*) smoothly afforded 2-phenyl-1-azapyrene in 70% yield. A solution of the base (0·36 g.) in anhydrous nitrobenzene (1·5 c.c.) was heated to 150—160° and then treated with methyl sulphate (0·24 c.c.). The mixture was heated at 150—160° for 5 minutes, cooled, and then diluted with anhydrous ether. The crystalline product was collected and dissolved in warm water (5 c.c.). The solution was filtered and then treated with saturated potassium bromide solution. The precipitate, on recrystallisation from water, formed clusters of yellow needles (0·38 g.), m. p. 227° (decomp.), of 2-*phenyl*-1-*azapyrene methobromide dihydrate* (Found: C, 64·6; H, 5·2; N, 3·6. $C_{23}H_{16}NBr,2H_2O$ requires C, 64·4; H, 4·9; N, 3·4%). 4-Carbethoxyaminobenzamido-, 4-*p*-nitrobenzamido-, and 2-methoxy-5-*p*-nitrobenzamido-phenanthrene were largely unaffected by similar treatment with phosphoric oxide in boiling xylene. In the case of the last named, a very low yield of 4-*methoxy*-2-p-*nitrophenyl*-1-*azapyrene* (II; R = OMe; R′ = NO_2), minute, yellow crystals (from acetic acid), m. p. 259—260° (Found: N, 7·9. $C_{23}H_{14}O_3N_2$ requires N, 7·9%), was isolated. The carbethoxyamino-compound was also largely unchanged on treatment with phosphoric oxide in syrupy phosphoric acid according to Bailey and Robinson's method (*J.*, 1950, 1375). Attempts to cyclise derivatives of 4-benzamidophenanthrene with phosphoryl chloride alone also met with little success.

Action of Phosphoryl Chloride on 2-Methoxy-5-p-nitrobenzamidophenanthrene.—The amide (100 mg.) was heated under reflux on a water-bath with phosphoryl chloride (1 c.c.) for 2 hours. The resulting solution was evaporated at 40° under reduced pressure. The residue was treated with ice and dilute ammonia solution. The resulting orange, heterogeneous solid (100 mg.) was dissolved in warm acetone. Addition of an acetone solution of picric acid gave a precipitate (20 mg.), which, on repeated recrystallisation from acetone, formed minute, orange prisms, m. p. 243—246° (decomp.), of 4-*methoxy*-2-p-*nitrophenyl*-1-*azapyrene picrate* (Found: C, 57·4; H, 3·3; N, 11·6. $C_{23}H_{14}O_3N_2,C_6H_3O_7N_3$ requires C, 57·6; H, 3·0; N, 12·0%).

Action of Phosphoryl Chloride on 4-Carbethoxyaminobenzamidophenanthrene.—(i) A solution of the amide (200 mg.), in phosphoryl chloride (2 c.c.), was boiled under reflux on an oil-bath for 60 minutes. It was then evaporated at 40° under reduced pressure. The residual yellow syrup, on trituration with ice-water, yielded an orange, sticky solid which on treatment with ice-cold, N-ammonia largely dissolved. The solution was filtered and then cautiously acidified with dilute hydrochloric acid. The precipitate (120 mg.; m. p. 125—140°) was extracted with warm ethanol. The extract was concentrated under reduced pressure and then diluted with water. The yellow precipitate (40 mg.), on repeated recrystallisation from methanol, formed sheaves of pale yellow prisms, m. p. 96—98° (decomp.), of an unidentified *substance* whose analytical data approached those of 2-*p*-carboxyaminophenyl-1-azapyrene (Found: C, 77·5; H, 4·4; N, 8·0. $C_{23}H_{14}O_2N_2$ requires C, 78·0; H, 4·2; N, 8·3%).

(ii) The amide (630 mg.) was boiled under reflux (oil-bath) with phosphoryl chloride (3·1 c.c.) for 6 hours. Excess of phosphoryl chloride was removed at 40° under reduced pressure and the residue treated with crushed ice. The mixture was filtered. Basification of the aqueous filtrate with dilute aqueous ammonia gave a yellow precipitate (0·28 g.; m. p. 120—130°), which was extracted with boiling ethanol. Concentration of the extract, followed by treatment of it with ethanolic picric acid, yielded a precipitate (0·4 g.) which, on repeated recrystallisation from ethanol, formed microscopic, orange prisms, m. p. 216—219° (decomp.), of 2-p-*aminophenyl*-1-*azapyrene picrate* (Found: C, 61·7; H, 3·3; N, 13·0. $C_{21}H_{14}N_2,C_6H_3O_7N_3$ requires C, 62·0; H, 3·3; N, 13·4%). The picrate was decomposed by treatment with dilute ammonia solution and the base extracted into chloroform. The extract was evaporated and the residual gum purified by adsorption on a column of alumina from benzene solution. The pale yellow band formed was eluted by 0·2% methanol–benzene and, on repeated recrystallisation from methanol, then yielded minute, yellow rhombs, m. p. 124—125°, of 2-p-*aminophenyl*-1-*azapyrene monohydrate* (II; R = H; R′ = NH_2) (Found: C, 81·3; H, 4·9; N, 9·2. $C_{21}H_{14}N_2,H_2O$ requires C, 80·7; H, 5·2; N, 9·0%). No uniform material could be isolated from the hydrochloric acid-insoluble products of the reaction.

2-p-*Nitrophenyl*-1-*azapyrene* (II; R = H; R′ = NO_2).—4-*p*-Nitrobenzamidophenanthrene (0·7 g.) was heated under reflux with phosphoryl chloride (2 c.c.) and anhydrous nitrobenzene (5 c.c.) in an oil-bath at 155—160° for 2 hours. The mixture was cooled, decomposed with crushed ice, and then distilled in steam. The residual solid was collected and recrystallised from ethanol (charcoal), followed by acetic acid, to give the *azapyrene* as yellow needles (0·4 g.), m. p. 241° (Found: N, 8·4. $C_{21}H_{12}O_2N_2$ requires N, 8·6%).

2-p-*Aminophenyl*-1-*azapyrene Metholuene*-p-*sulphonate.*—A solution of the foregoing nitro-azapyrene (0·35 g.) in anhydrous nitrobenzene was heated to 180° and then treated portionwise with methyl toluene-*p*-

sulphonate (0·4 g.). The mixture was heated at 200° for 15 minutes. The crystalline material which separated on cooling was filtered off, washed with ether, and recrystallised from water to give long, yellow needles (0·32 g.), m. p. 245—250°. These were heated under reflux on a water-bath with " reduced " iron powder (0·35 g.), 0·1N-hydrochloric acid (1 c.c.), and ethanol (10 c.c.) for 1·5 hours. The mixture was treated with 0·1N-sodium hydroxide (0·8 c.c.) and ethanol (15 c.c.), and filtered while hot. Concentration of the filtrate gave the amino-quaternary *salt* in crimson plates (0·2 g.), m. p. 268—270° (decomp.) (Found : C, 72·8; H, 5·0; N, 6·0. $C_{39}H_{44}O_3N_2S$ requires C, 72·5; H, 5·0; N, 5·8%).

4-Carbethoxyamino-2-p-nitrophenyl-1-azapyrene Methosulphate.—2-Carbethoxyamino-5-*p*-nitrobenzamidophenanthrene (1·32 g.) was heated under reflux with anhydrous nitrobenzene (12 c.c.) at 135—140° (bath-temp.) and treated dropwise with phosphoryl chloride (2·6 c.c.). The mixture was heated at 135—140° for 1 hour, cooled, poured into water, basified with concentrated ammonia solution, and then distilled in steam. The residual solid was best purified at the next stage. It was heated with anhydrous nitrobenzene (6 c.c.) at 155—165° and treated with methyl sulphate (0·6 c.c.). The mixture was heated at 160—165° for 8 minutes, cooled, poured into water, and then distilled in steam. The residue was concentrated to approx. 80 c.c and then filtered while hot. The *methosulphate sesquihydrate* separated from the filtrate in minute, yellow prisms (0·58 g.), m. p. 230—232° (decomp.) (Found : C, 55·3; H, 4·8; N, 7·3. $C_{26}H_{23}O_6N_3S,1\frac{1}{2}H_2O$ requires C, 55·3; H, 4·6; N, 7·4%).

2-p-*Aminophenyl-4-carbethoxyamino-1-azapyrene Methosulphate.*—The foregoing nitro-quaternary salt (280 mg.) was added, in portions, to a mixture of " reduced " iron powder (300 mg.), 0·05N-sulphuric acid (2 c.c.), and ethanol (6 c.c.), heated under reflux on a water-bath. The mixture was boiled under reflux for 2 hours, treated with 0·1N-sodium hydroxide (0·9 c.c.), then filtered while hot. The residue was extracted with several portions of boiling ethanol. The combined filtrates were concentrated to approx. 5 c.c. and set aside for several hours. The red solution was decanted from dark red gum and evaporated. The residue, on being rubbed with a small quantity of methanol, yielded a solid which, on repeated recrystallisation from methanol, formed long, scarlet needles (80 mg.), m. p. 225° (decomp.), of the amino-quaternary salt *monohydrate* (Found : C, 59·3; H, 5·4; N, 7·6. $C_{26}H_{25}O_6N_3S,H_2O$ requires C, 59·4; H, 5·2; N, 8·0%).

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549. *The Basic Strengths of Methylated Guanidines.*

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All the possible methyl-substituted guanidines have been prepared and their dissociation constants measured. Contrary to a previous report, they are all very strong bases.

AN interesting anomaly in the base strengths of substituted guanidines was reported by Davis and Elderfield (*J. Amer. Chem. Soc.*, 1932, **54**, 1499). They found that, like the parent substance, the *N*-methyl, *NN*-dimethyl-, and *NN'N''*-trimethyl derivatives were bases comparable in strength with the inorganic hydroxides, but the *NN'*-dialkylguanidines were much weaker, having a pK_a of about 10·3. Pauling (" The Nature of the Chemical Bond," 1940, p. 213) has attempted an explanation of this anomaly in terms of the resonance theory; he predicted that the *NNN'N'*-tetramethyl derivative should be an even weaker base (Gilman, " Organic Chemistry," 1943, Vol. 2, p. 1966). Referring to his arguments, Wheland (" The Theory of Resonance," 1944, p. 180) commented : " An ingenious explanation of these facts has been given by Pauling, but the problem might repay further experimental investigation." This investigation has now been carried out with the surprising result that the anomaly to be explained does not exist at all.

To obtain as much information as possible on the effect of methyl substitution on guanidines, the eight methylated guanidines have been prepared and their pK_a values determined. Difficulties were experienced in some of the preparations. All the guanidines have been synthesised before, mainly by the life-long work of Schenk (*Arch. Pharm.*, 1909, **247**, 466, 490; *Z. physiol. Chem.*, 1912, **77**, 328; 1925, **150**, 121; Schenk and Graevenitz, *ibid.*, 1924, **141**, 132; Schenk and Kirchhof, *ibid.*, 1926, **153**, 150), but the more highly substituted ones were isolated only as chloroplatinates, chloroaurates, or picrates. Our attempts to convert the picrates into other salts were not successful; we therefore evolved methods for the direct preparation of simple salts. The reaction of the appropriate thiuronium salts with ammonia, methylamine, or dimethylamine provided unambiguous syntheses for every methylated guanidine.

Experimental.

M. p.s are corrected. Microanalyses by E. Bielski.

Preparation of the Guanidines.—Methylguanidinium sulphate, m. p. 240°, and *NN*-dimethylguanidinium sulphate, m. p. 295—297°, were prepared by Clarke and Phillips's method (*J. Amer. Chem. Soc.*, 1923, **45**, 1755) in 82 and 75% yield from *S*-methylthiuronium sulphate by the action of methylamine and dimethylamine, respectively. Clarke and Phillips report m. p.s 239° and 285—288° respectively. In view of our higher m. p. for the dimethyl compound, its identity was established by the preparation of the picrate, m. p. 224—225°; Davis and Elderfield (*loc. cit.*) report m. p. 224°.

Attempts were made to extend this method to the higher homologues but were abandoned because no crystalline thiuronium sulphates could be obtained from any of the methylated thioureas by treatment with methyl sulphate. The action of methyl iodide, however, yielded well-characterised thiuronium salts from which the guanidines were obtained as the hydriodides. Their crystallisation from the oily reaction mixtures was difficult and was achieved only by careful treatment with anhydrous ethanol and ether. Once crystalline, although mostly very hygroscopic, they recrystallised readily.

NN'-Dimethylguanidine.—*SNN'*-Trimethylthiuronium iodide (4·2 g.) (Schenk, *loc. cit.*, 1912) was heated at 65—75° for 2 hours with ethanolic ammonia (6·0 c.c.; 10%). Methanethiol was evolved. The reaction mixture was evaporated to dryness under reduced pressure and the residual oil was dissolved in anhydrous ethanol. Addition of ether gave a crop of ammonium iodide, and the mother-liquor, on addition of more ether, deposited an oil which gradually solidified. Two recrystallisations from ethanol–ether gave deliquescent plates of the *hydriodide*, m. p. 103·5—105° (22%) (Found: N, 19·55. $C_3H_{10}N_2I$ requires N, 19·55%).

The same compound was also prepared by the following method (cf. Schenk, *loc. cit.*, 1909): Freshly sublimed cyanogen iodide (7·6 g.) was dissolved in anhydrous ethanolic methylamine solution (45 c.c.; 10%). The reaction vessel was firmly stoppered and set aside for 48 hours. The initially brown mixture was then colourless. Removal of the solvent under reduced pressure yielded a crop of crystals which were recrystallised three times from anhydrous ethanol–ether to give the hydriodide (5·4 g., 48%), m. p. 104—105·5°.

By treatment with silver nitrate, *NN'*-dimethylguanidinium iodide was converted into the *nitrate*, m. p. 102—104° (from aqueous ethanol) (Found: N, 37·3. $C_3H_{10}O_3N_4$ requires N, 37·3%). When shaken with silver sulphate in water, the hydriodide gave the *sulphate*, m. p. 298—300° (decomp.) (from water) (Found: N, 30·65. $C_6H_{20}O_4SN_6$ requires N, 30·85%). The picrate melted at 178—179°; Schenk (*loc. cit.*, 1912) reports m. p. 178°. The same picrate was also obtained from the reaction of *SN*-dimethylthiuronium iodide with methylamine, but the hydriodide did not crystallise.

NNN'-Trimethylguanidine.—*SN*-Dimethylthiuronium iodide (23·5 g.) (Schenk, *loc. cit.*, 1912) and aqueous dimethylamine (60 c.c.; 16%) were heated at 100° for 3 hours and then worked up as above to give the *hydriodide* (8·7 g., 41%), m. p. 202·5—204° (Found: N, 18·25. $C_4H_{12}N_2I$ requires N, 18·35%). The picrate, m. p. 151—151·5°, crystallised from ethanol (Found: N, 25·35. Calc. for $C_{10}H_{14}O_7N_6$: N, 25·45%) (Schenk and Kirchhof, *loc. cit.*, m. p. 151—152°).

NN'N''-Trimethylguanidine.—*SNN'*-Trimethylthiuronium iodide (22·0 g.) and aqueous methylamine solution (18 c.c.; 30%), worked up as above, gave the hydriodide (14·8 g., 73%), recrystallised from a large volume of alcohol, m. p. >300° (Found: N, 18·35%). The sulphate, m. p. >300°, was obtained in nearly quantitative yield by shaking this salt with silver sulphate and crystallisation from aqueous ethanol. The picrate melted at 214—215° (Davis and Elderfield, *loc. cit.*, 214·5°).

NNN'N''-Tetramethylguanidine.—*SNNN'*-Tetramethylthiuronium iodide (30 g.) (obtained as a glass, Schenk, *loc. cit.*, 1912) and ethanolic methylamine solution (27 c.c.; 30%) were heated for 1 hour on the water-bath and worked up as above to give the *hydriodide* (13·0 g., 46%), m. p. 120—120·5° (Found: N, 17·15. $C_5H_{14}N_2I$ requires N, 17·3%). Schenk, using higher temperatures, did not obtain this guanidine by this method. The picrate melted at 159—160° (Schenk, 158—160°).

NNN'N'-Tetramethylguanidine.—Pentamethylthiuronium iodide (20·8 g.) (Delépine, *Bull. Soc. chim.*, 1910, **7**, 992), dissolved in the minimum amount of water, was warmed for 2 hours at 100° with concentrated ammonia solution (20 c.c.). The mixture was worked up as above, to give the *hydriodide* (7·5 g., 40%), hygroscopic crystals, m. p. 131—131·5° (Found: N, 17·0%). The picrate melted at 130—131°; Schenk (*loc. cit.*, 1912) reports 130°.

Pentamethylguanidine.—Pentamethylthiuronium iodide (24 g.) and methylamine (7·0 g.) in anhydrous ethanol (50 c.c.) were heated to 65—70° for 2 hours and worked up as above, to give the *hydriodide* (5·2 g., 21%), m. p. 136—138° (Found: C, 30·1; H, 6·65; N, 15·0. $C_6H_{16}N_2I,\frac{1}{4}C_2H_5OH$ requires C, 30·0; H, 6·8; N, 15·0%). The picrate melted at 163—165°; Lecher and Graf (*Annalen*, 1924, **438**, 154) have also described the hydriodide but did not record its m. p.; for the picrate they report (*Ber.*, 1923, **56**, 1329) m. p. 165—166° (corr.).

pK$_a$ Measurements.—The dissociation constants were measured by potentiometric titration, following closely, in experimental details and in calculation, Hall and Sprinkle's determination of the pK_a of guanidine (*J. Amer. Chem. Soc.*, 1932, **54**, 3475), except that a glass electrode ("Alki," Cambridge Instrument Co.) was used instead of a hydrogen electrode. The pH values were determined with a Leeds and Northrup Universal pH Potentiometer Assembly (No. 7663-A-1) adjusted to glycine buffers of pH 11·14 and 12·66. Hall and Sprinkle titrated a 2N-solution but, some of our compounds being insufficiently soluble, we adopted N-solutions for all the measurements, with some loss of accuracy. *NN'N''*-Trimethylguanidinium iodide would not even give a N-solution but the sulphate was sufficiently soluble. The solutions were made up in carbon dioxide-free water and were titrated in a stream of nitrogen. Corrections were not applied for the potassium-ion error of the glass electrode, or for the

hydrogen-ion activity coefficients because reliable data were not available for these corrections. In view of these facts, and of the high pH involved, the results are not regarded as very accurate: the error may be as high as 0·2 pH unit.

Discussion.

The experimental results are recorded in the Table. The pK_a value of guanidine agrees well with Hall and Sprinkle's more accurately measured and corrected value of 13·6$_3$ (*loc. cit.*). The results listed in the Table show that all the methylated guanidines are very strong bases, the base strengths being only slightly affected by methyl substitution. The symmetrical dimethyl derivative is no exception. There seems to be little doubt that our compound was really *NN'*-dimethylguanidine: it was prepared in two different ways and three of its salts (sulphate, nitrate, and iodide) gave correct analyses. The high pK_a value in itself indicates that the compound is a guanidine. But it seems also certain that Davis and Elderfield's compound was not identical with our *NN'*-dimethylguanidine: not only their pK_a value but also the melting points of their sulphate (264—265°) and nitrate (68°) differed widely from ours (298—300° and 102—104°, respectively). We conclude therefore that the compound of which Davis and Elderfield reported the base strength was not *NN'*-dimethylguanidine.

From the scanty details given by the American authors we were not able to establish the identity of their compound. They state that it was prepared by Kaess and Gruszkiewicz's method (*Ber.*, 1902, **35**, 3599). These authors obtained, by the reaction of cyanogen chloride and methylamine in dry ether, a solid, of no definite melting point, which gave correct analysis figures for a *di*hydrochloride of dimethylguanidine. This in itself throws serious doubt on the identity of their compound for guanidines are theoretically not expected, and have never been found, to be diacid bases. Methylamine hydrochloride would undoubtedly be precipitated in this reaction and would have been part of their product; the rest might have been methylcyanamide hydrochloride. Davis and Elderfield give no indication of how this product was converted into a nitrate and a sulphate, and we were not able therefore to repeat their preparation. The possibility that their salts were molecular addition compounds of methylamine salts and methylcyanamide was investigated, but we were unable to prepare crystalline products by mixing these ingredients.

Guanidine.	Salt used.	pK_a at 25°.
(Unsubstituted)	Sulphate	13·6
N-Methyl	,,	13·4
NN-Dimethyl	,,	13·4
NN'-Dimethyl	Iodide	13·6
NNN'-Trimethyl	,,	13·6
NN'N''-Trimethyl	Sulphate	13·9
NNN'N'-Tetramethyl	Iodide	13·6
NNN'N''-Tetramethyl	,,	13·9
Pentamethyl	,,	13·8

The fact that all the pK_a values were measured under identical conditions makes it probable that, even if the absolute values do not differ by much more than the experimental error, the *relative* values are subject to less error. We therefore regard the differences between the pK_a values as significant and feel justified in discussing them. The variations are in the expected directions. Methyl groups are base-strengthening but a substitution which destroys the equivalence of the three nitrogens in guanidine will be base-weakening because it reduces the resonance energy of the ion. It is not surprising therefore that the introduction of one methyl group lowers the pK_a value of guanidine. The combined effect of several methyl groups compensates for this effect but it is notable that in each pair of isomers the one with the more even distribution of substituents is the stronger base.

University of Sydney, N.S.W., Australia. [*Received, May 23rd*, 1951.]

550. *Reactions of Fluorocarbon Radicals. Part V.* Alternative Syntheses for Trifluoromethylacetylene (3 : 3 : 3-Trifluoropropyne), and the Influence of Polyfluoro-groups on Adjacent Hydrogen and Halogen Atoms.*

By R. N. Haszeldine.

Syntheses of trifluoromethylacetylene from trifluoroacetic acid or trifluoroiodomethane by preparation and dehydrohalogenation or dehalogenation of compounds of the type $CF_3{\cdot}CH_2{\cdot}CHXY$, $CF_3{\cdot}CH{:}CHX$, $CF_3{\cdot}CX_2{\cdot}CHY_2$, $CF_3{\cdot}CX{:}CH_2$, and $CF_3{\cdot}CX{:}CHY$ (X or Y = halogen other than fluorine) have been investigated. 3 : 3 : 3-Trifluoropropene has proved to be the most convenient intermediate for the preparation of comparatively large amounts of the acetylene. The influence of the trifluoromethyl group on the reactivity of hydrogen and halogen atoms attached directly to it, attached to an adjacent carbon atom, and attached to the carbon atom once removed is considered. The infra-red spectrum of trifluoromethylacetylene is reported.

3 : 3 : 3-Trifluoropropyne was first prepared by the interaction of trifluoroiodomethane and acetylene to give 3 : 3 : 3-trifluoro-1-iodopropene, followed by dehydroiodination (Haszeldine, *Nature*, 1950, **165**, 152; *J.*, 1951, 588). The reaction of trifluoroiodomethane with acetylene can be carried out in glass apparatus at pressures of 10—15 atmospheres, and although it constitutes a convenient synthesis for small laboratory-scale quantities of the trifluoromethylacetylene, the dangers associated with acetylene under pressure prevent its convenient application on a large scale. Other syntheses have now been developed, some of which are suitable for the preparation of larger quantities.

Trifluoroacetic acid was used as the source of the CF_3 group, and was converted into trifluoroiodomethane by reaction of the silver salt with iodine (Haszeldine, *Nature*, 1950, **166**, 192; *J.*, 1951, 584). Trifluoroiodomethane, also prepared from carbon tetraiodide (Haszeldine, *J.*, 1950, 3037), was converted by reaction with ethylene into a polymer $CF_3{\cdot}[CH_2{\cdot}CH_2]_n{\cdot}I$, in which the compound with $n = 1$ predominated (Haszeldine, *J.*, 1949, 2856).

$$CF_3{\cdot}CO_2H \longrightarrow CF_3{\cdot}CO_2Ag \downarrow$$
$$CI_4 \longrightarrow CF_3I \longrightarrow CF_3{\cdot}CH_2{\cdot}CH_2I$$

The synthesis of trifluoromethylacetylene from 3 : 3 : 3-trifluoro-1-iodopropane can be performed by four routes.

Route I.—The fluoroiodopropane is converted into a compound of general formula $CF_3{\cdot}CH_2{\cdot}CHXY$ (X or Y = Cl, Br, or I), which, on dehydrohalogenation, yields trifluoromethylacetylene. The following reaction schemes were employed for the preparation of $CF_3{\cdot}CH_2{\cdot}CHClI$, $CF_3{\cdot}CH_2{\cdot}CHBrI$, $CF_3{\cdot}CH_2{\cdot}CHCl_2$, $CF_3{\cdot}CH_2{\cdot}CHBr_2$, and $CF_3{\cdot}CH_2{\cdot}CHClBr$:

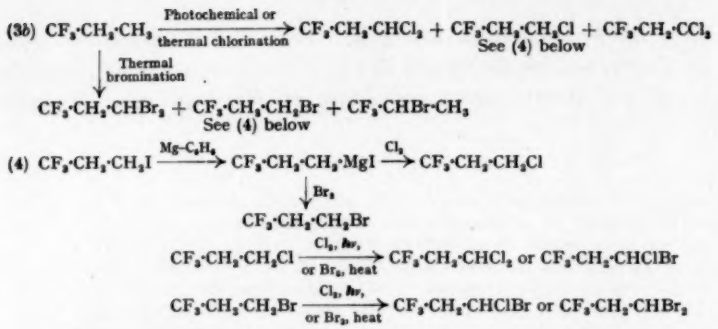
(1) $CF_3{\cdot}CH_2{\cdot}CH_2I \xrightarrow[\text{chlorination}]{\text{Photochemical}} \underset{\text{Main product}}{CF_3{\cdot}CH_2{\cdot}CHClI} + \underset{\text{See (4) below}}{CF_3{\cdot}CH_2{\cdot}CH_2Cl} + CF_3{\cdot}CH_2{\cdot}CHCl_2 + CF_3{\cdot}CH_2{\cdot}CCl_3$

(2) $CF_3{\cdot}CH_2{\cdot}CH_2I \xrightarrow[\text{bromination}]{\text{Thermal}} CF_3{\cdot}CH_2{\cdot}CHBrI + \underset{\text{See (4) below}}{CF_3{\cdot}CH_2{\cdot}CH_2Br} + CF_3{\cdot}CH_2{\cdot}CHBr_2$

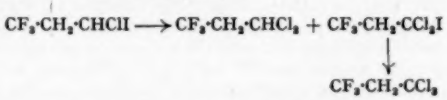
(3*a*) $CF_3{\cdot}CH_2{\cdot}CH_2I \xrightarrow{Mg\text{-}Et_2O} CF_3{\cdot}CH_2{\cdot}CH_2{\cdot}MgI \xrightarrow{H_2O} CF_3{\cdot}CH_2{\cdot}CH_3$

$CF_3{\cdot}CH_2{\cdot}CH_2I \xrightarrow{RH\text{-}Hg} CF_3{\cdot}CH_2{\cdot}CH_3$

* Part IV, *J.*, 1951, 588.



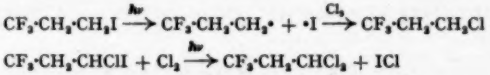
Scheme 1. Photochemical chlorination of 1 : 1 : 1-trifluoro-3-iodopropane gave, under the conditions used, mainly 3-chloro-1 : 1 : 1-trifluoro-3-iodopropane. The structural formula of this product follows from the facts that treatment with zinc and alcohol does not give trifluoropropene and that irradiation with chlorine yields 3 : 3-dichloro-1 : 1 : 1-trifluoropropane and 3 : 3-dichloro-1 : 1 : 1-trifluoro-3-iodopropane. The structural formula of the last compound is, in turn, shown by the fact that irradiation with chlorine in a Pyrex vessel yields only one product, 3 : 3 : 3-trichloro-1 : 1 : 1-trifluoropropane :

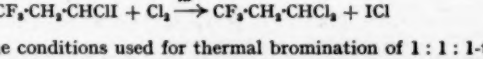


The trichlorotrifluoropropane can be identified by its boiling point, which is appreciably different from that of its isomer 2 : 3 : 3-trichloro-1 : 1 : 1-trifluoropropane, and by the fact that treatment with zinc and alcohol does not give 1-chloro-3 : 3 : 3-trifluoropropene.

The structural formula of 3 : 3-dichloro-1 : 1 : 1-trifluoropropane formed by chlorination of 1 : 1 : 1-trifluoro-3-iodopropane and of 3-chloro-1 : 1 : 1-trifluoro-3-iodopropane rests partly on its physical properties, but mainly on the facts that further chlorination gives 3 : 3 : 3-trichloro-1 : 1 : 1-trifluoropropane, and that treatment with zinc and alcohol does not give 3 : 3 : 3-trifluoropropene. The formula of 3 : 3 : 3-trichloro-1 : 1 : 1-trifluoropropane isolated from 1 : 1 : 1-trifluoro-3-iodopropane and chlorine is based upon similar arguments, and that of the fourth product 3-chloro-1 : 1 : 1-trifluoropropane by its boiling point which is 15° higher than that of the possible isomer 2-chloro-1 : 1 : 1-trifluoropropane; furthermore, the formation of the 2-chloro-compound by the chlorination of the 3-iodo-compound is unlikely.

It is evident, therefore, that the chlorination of 1 : 1 : 1-trifluoro-3-iodopropane proceeds by replacement of the hydrogen atom on the carbon carrying the iodine atom, and that this is accompanied either by homolytic fission of the carbon–iodine bond or by replacement of iodine in an iodo-compound by chlorine, *e.g.* :





Scheme 2. The conditions used for thermal bromination of 1 : 1 : 1-trifluoro-3-iodopropane were more stringent than those for its chlorination, and products (*e.g.*, bromotrifluoromethane) other than those indicated were also formed by decomposition. In general, however, the bromination resembled the chlorination and yielded 3-bromo-1 : 1 : 1-trifluoro-3-iodopropane, whose structure was established by photochemical bromination at room temperature to give 3 : 3-dibromo-1 : 1 : 1-trifluoropropane. The boiling point of the latter compound is sufficiently removed from that of the isomer 2 : 3-dibromo-1 : 1 : 1-trifluoropropane to make identification possible. In addition, it was found that dehydrobromination was readily effected and in good yield, as was observed with the 3 : 3-dibromo-compound prepared by a different route, and that heating with zinc and alcohol did not yield 3 : 3 : 3-trifluoropropene.

Other compounds identified in the product from the thermal bromination of 1 : 1 : 1-trifluoro-3-iodopropane were 1 : 1 : 1-trifluoro-3 : 3-dibromo- and -3-bromo-propane whose boiling point is some 13° higher than that of the 2-bromo-isomer.

Scheme 3. The fluoropropane, $CF_3{\cdot}CH_2{\cdot}CH_3$, was prepared from 1 : 1 : 1-trifluoro-3-iodopropane by reaction in ultra-violet light at room temperature, or at high temperature in an autoclave, with a compound from which the free $CF_3{\cdot}CH_2{\cdot}CH_2$ radicals are capable of abstracting hydrogen atoms. Such compounds are alcohols, ethers, and hydrocarbons, but to avoid side reactions hydrocarbons are preferred. The addition of mercury to remove iodine and so prevent primary recombination facilitates the reaction (Haszeldine, *J.*, 1949, 2856). Small amounts of 3 : 3 : 3-trifluoropropene, possibly formed by disproportionation $2CF_3{\cdot}CH_2{\cdot}CH_2{\cdot} \longrightarrow CF_3{\cdot}CH_2{\cdot}CH_3 + CF_3{\cdot}CH{:}CH_2$ were also identified. A more suitable method for the conversion of 1 : 1 : 1-trifluoro-3-iodopropane into trifluoropropane is by formation and hydrolysis of the Grignard reagent, $CF_3{\cdot}CH_2{\cdot}CH_2{\cdot}MgI$ (forthcoming communication).

The reactions illustrated by scheme (3*b*), *i.e.*, the conversion of trifluoropropane into 3 : 3-dichloro- and 3 : 3-dibromo-1 : 1 : 1-trifluoropropane by chlorination or thermal bromination, were first carried out by Henne and by McBee with their respective co-workers. Somewhat different experimental conditions have been used in the present work and the results are now discussed. Henne and Whaley (*J. Amer. Chem. Soc.*, 1942, **64**, 1157) observed that photochemical chlorination of 1 : 1 : 1-trifluoropropane gave 3-chloro-, 3 : 3-dichloro-, and 3 : 3 : 3-trichloro-1 : 1 : 1-trifluoropropane, and intermediate fractions in a weight ratio of 21 : 41 : 26 : 12. Photochemical chlorination at room temperature by the method described in the Experimental section gave a product which contained the above fractions in a weight ratio of 16 : 62 : 14 : 8. Thus, it is possible to chlorinate trifluoropropane to give mainly the mono- and di-chloro-compounds. In accord with Henne and Whaley's results, a negligible amount of the 2-chloro-trifluoropropane was formed. Vapour phase photochemical chlorination at 80—100°, however, gave an appreciable amount of the 2-chloro-1 : 1 : 1-trifluoropropane. The weight ratio of 3-chloro-, 2-chloro-, 3 : 3-dichloro-, and 3 : 3 : 3-trichloro-1 : 1 : 1-trifluoropropane was 55 : 18 : 17 : 2 which is in substantial agreement with McBee and Truchan's results (*J. Amer. Chem. Soc.*, 1948, **70**, 2910), who found a ratio of 58 : 22 : 17 : *x* (not quoted), by a flow-chlorination technique. Thermal chlorination (420°) of trifluoropropane gave a weight ratio of products of 36 : 3 : 18 : 8, which can be compared with the figures 44 : 12 : 11 : (unknown) calculated from the results of McBee, Hass, Thomas, Toland, and Truchan (*J. Amer. Chem. Soc.*, 1947, **69**, 944) for a similar temperature (380°). The amount of the 2-chloro-1 : 1 : 1-trifluoropropane formed clearly depends on the technique and conditions used. The structural formulæ of the products of chlorination were established by the procedures outlined earlier.

Thermal bromination of trifluoropropane gave 3 : 3-dibromo-1 : 1 : 1-trifluoropropane and two isomeric monobromo-compounds, $CF_3{\cdot}CH_2{\cdot}CH_2Br$ and $CF_3{\cdot}CHBr{\cdot}CH_3$. The amount of the 2-bromo-compound isolated was much less than was found by McBee, Hass, Toland, and Truchan (*Ind. Eng. Chem.*, 1947, **39**, 420) (cf. weight ratios of 3-bromo- : 2-bromo- : 3 : 3-dibromo-compound, 30 : 6 : 64 and 43 : 25 : 32 respectively).

Scheme 4. The 3-bromo- and 3-chloro-1 : 1 : 1-trifluoropropanes produced as by-products in the earlier reactions could also be obtained from the 3-iodo-compound by interaction of the Grignard reagent and bromine or chlorine, and from ethylene by free-radical addition of carbon tetrachloride or bromotrichloromethane to give $CCl_3{\cdot}CH_2{\cdot}CH_2Cl$ or $CCl_3{\cdot}CH_2{\cdot}CH_2Br$, followed by catalysed antimony fluoride fluorination (unpublished). Chlorination or bromination converted these 3-halogeno-fluoropropanes into $CF_3{\cdot}CH_2{\cdot}CHCl_2$, $CF_3{\cdot}CH_2{\cdot}CHBr_2$, or $CF_3{\cdot}CH_2{\cdot}CHClBr$. The 3 : 3-dihalogeno-compounds were formed in good yield by chlorination of the monohalogeno-compounds, whereas thermal bromination brought about some decomposition. The formulæ of the first two compounds were established as described earlier. That of 3-bromo-3-chloro-1 : 1 : 1-trifluoropropane was based on its failure to give 3 : 3 : 3-trifluoropropene with zinc and alcohol (although it was not inert to this reagent), and on its dehydrohalogenation to 1-chloro-3 : 3 : 3-trifluoropropene and trifluoromethylacetylene.

Photochemical chlorination of 3-chloro-1 : 1 : 1-trifluoropropane gave some 3 : 3 : 3-trichloro-1 : 1 : 1-trifluoropropane, and of the 3-bromo-compound gave a small amount of 3-bromo-3 : 3-dichloro-1 : 1 : 1-trifluoropropane in addition to the compounds noted above. The structure of the bromodichloro-compound was established by dehydrobromination to an olefin ($CF_3{\cdot}CH{:}CCl_2$) which was not dechlorinated by zinc and alcohol. The bromodichloro-isomers which could have been formed were $CF_3{\cdot}CCl_2{\cdot}CH_2Br$, which could not undergo dehydrobromination, and $CF_3{\cdot}CHCl{\cdot}CHClBr$. The last compound, on dehydrobromination, would

give an olefin ($CF_3 \cdot CCl{:}CHCl$), which has been found to give trifluoromethylacetylene in small yield when treated with zinc and alcohol.

It will be noted that the chlorination and bromination of $CF_3 \cdot CH_2 \cdot CH_2X$ is again markedly directed to the carbon atom which already carries the lone halogen X.

The compounds of formulæ $CF_3 \cdot CH_2 \cdot CH_2X$, $CF_3 \cdot CHX \cdot CH_3$, and $CF_3 \cdot CH_2 \cdot CHX_2$ obtained by the routes described above were dehydrohalogenated by powdered potassium hydroxide. It was found that reaction occurred most easily and with the best yields when the hydrogen removed was on the carbon atom adjacent to the CF_3 group, and the halogen atom was on the carbon atom once removed, *i.e.*, the most suitable system was $CF_3 \cdot \overset{|}{C}H \cdot \overset{|}{C}X$, and X was preferably iodine or bromine. Removal of hydrogen chloride from 2-chloro-1 : 1 : 1-trifluoropropane was achieved in only poor yield, and the chlorine atom is thus stabilised by its position on the carbon atom adjacent to the CF_3 group: the 2-bromo-compound gave only a slightly better yield of 3 : 3 : 3-trifluoropropene. The dehydrohalogenation of a compound such as 2 : 2-dichloro-1 : 1 : 1-trifluoropropane to give trifluoromethylacetylene was not attempted and, in view of the results with the 2-chloro-compound, is unlikely to be a suitable method for the preparation of the acetylene.

Route II.—The second method available for the preparation of trifluoromethylacetylene is by dehydrohalogenation of an olefin $CF_3 \cdot CH{:}CHX$ (X = Cl, Br, or I). The compound where X = I can be prepared from trifluoroiodomethane and acetylene, but the only convenient method at present available for the synthesis of the compounds where X = Cl and Br is from suitable $CF_3 \cdot CH_2 \cdot CHX_2$ compounds. The 1-bromo- and 1-chloro-3 : 3 : 3-trifluoropropenes were isolated as intermediates in the conversion of $CF_3 \cdot CH_2 \cdot CHCl_2$, $CF_3 \cdot CH_2 \cdot CHBr_2$, $CF_3 \cdot CH_2 \cdot CHClBr$, $CF_3 \cdot CH_2 \cdot CHBrI$, and $CF_3 \cdot CH_2 \cdot CHClI$ into trifluoromethylacetylene; the ease of hydrogen halide removal from these compounds is in the expected order HI > HBr > HCl. The structural formulæ of the halogenopropenes follow from the formulæ of the parent compounds: they were readily dehydrohalogenated to give the acetylene in good yield, in contrast to their isomers 2-bromo- and 2-chloro-3 : 3 : 3-trifluoropropene (see below).

Route III.—The third method for the synthesis of trifluoromethylacetylene was from compounds of general formula $CF_3 \cdot CXX' \cdot CHYY'$, by dehalogenation. Suitable compounds of this type have yet to be prepared. 2 : 2 : 3 : 3-Tetrachloro-1 : 1 : 1-trifluoropropane, for example, has been obtained only by addition of chlorine to trifluoromethylacetylene (Haszeldine, *J.*, 1951, 588). Unless X and X′ are bromine or iodine, the yields by this route will be poor, since the stabilising influence of the CF_3 group on X and X′ will render dehalogenation difficult.

Route IV.—The fourth method involves the conversion of the olefin 3 : 3 : 3-trifluoropropene into olefins of the type $CF_3 \cdot CX{:}CH_2$ or $CF_3 \cdot CX{:}CHY$, followed by dehydrohalogenation or dehalogenation. The 3 : 3 : 3-trifluoropropene was prepared from the compounds $CF_3 \cdot CH_2 \cdot CH_2X$ described earlier, but its preparation need not involve trifluoroiodomethane or trifluoroacetic acid, since $CF_3 \cdot CH_2 \cdot CH_2Cl$ and $CF_3 \cdot CH_2 \cdot CH_2Br$ can be obtained from 1 : 1 : 1 : 3-tetrachloro-, 3-bromo-1 : 1 : 1-trichloro- (above), or 1 : 1 : 1-trifluoro-propane which last, in turn, can be synthesised from 1 : 1 : 1-trichloropropane, 1 : 1-dichloropropene, or 1 : 1-dichloro*cyclo*propane (McBee, Hass, Thomas, Toland, and Truchan, *loc. cit.*).

The following syntheses were investigated:

(5*a*) $CF_3 \cdot CH{:}CH_2 \xrightarrow{Cl_2} CF_3 \cdot CHCl \cdot CH_2Cl \xrightarrow{KOH} CF_3 \cdot CCl{:}CH_2 \xrightarrow{KOH} CF_3 \cdot C{:}CH$

(5*b*) $CF_3 \cdot CCl{:}CH_2 \xrightarrow{Cl_2} CF_3 \cdot CCl_2 \cdot CH_2Cl \xrightarrow{KOH} CF_3 \cdot CCl{:}CHCl \xrightarrow{Zn\text{-}EtOH} CF_3 \cdot C{:}CH$

(5*c*) $CF_3 \cdot CH{:}CHCl \xrightarrow{Cl_2} CF_3 \cdot CHCl \cdot CHCl_2 \xrightarrow{KOH} CF_3 \cdot C{:}CH$

(6*a*) $CF_3 \cdot CH{:}CH_2 \xrightarrow{Br_2} CF_3 \cdot CHBr \cdot CH_2Br \xrightarrow{KOH} CF_3 \cdot CBr{:}CH_2 + CF_3 \cdot C{:}CH$

$CF_3 \cdot CBr{:}CH_2 \xrightarrow{KOH} CF_3 \cdot C{:}CH$

(6*b*) $CF_3 \cdot CBr{:}CH_2 \xrightarrow{Br_2} CF_3 \cdot CBr_2 \cdot CH_2Br \xrightarrow{KOH} CF_3 \cdot CBr{:}CHBr \xrightarrow{Zn\text{-}EtOH} CF_3 \cdot C{:}CH$

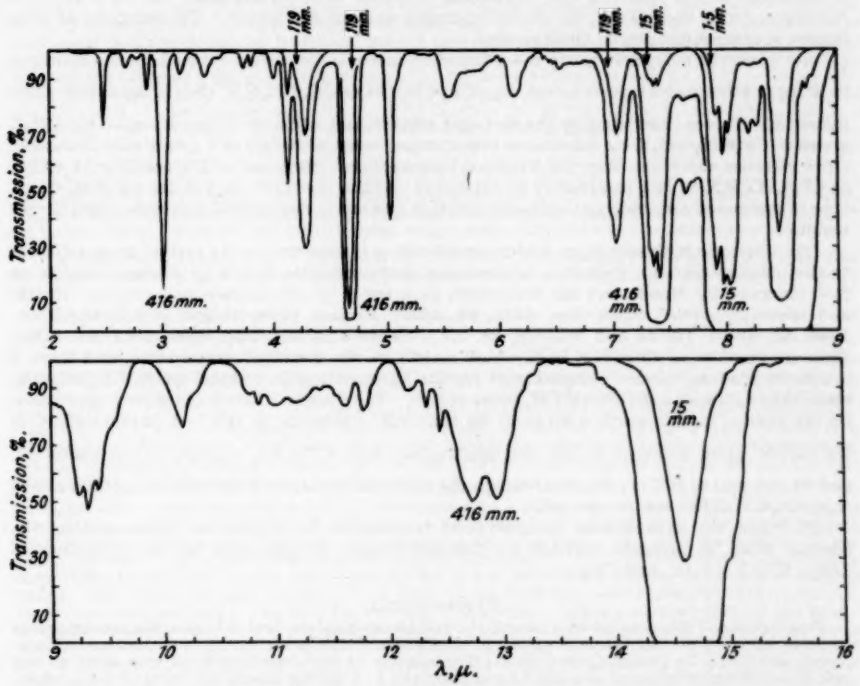
(6*c*) $CF_3 \cdot CH{:}CHBr \xrightarrow{Br_2} CF_3 \cdot CHBr \cdot CHBr_2 \xrightarrow{KOH} CF_3 \cdot C{:}CH$

The photo-catalysed addition of chlorine to 3 : 3 : 3-trifluoropropene gave 2 : 3-dichloro-1 : 1 : 1-trifluoropropane in good yield, but treatment with potassium hydroxide gave mainly a monochloro-trifluoropropene, which, on further treatment with alkali, gave the acetylene

in only poor yield. The two $C_3H_2ClF_3$ isomers thus differ greatly in their reactivity to alkali. The structural formula of the $CF_3{\cdot}CH{:}CHCl$ isomer is known by its synthesis from $CF_3{\cdot}CH_2{\cdot}CHX_2$ compounds. It thus follows that the other isomer (obtained from $CF_3{\cdot}CHCl{\cdot}CH_2Cl$) is $CF_3{\cdot}CCl{:}CH_2$, that in the dehydrochlorination of $CF_3{\cdot}CHCl{\cdot}CH_2Cl$ it is the hydrogen atom on thec arbon atom adjacent to the CF_3 group which is removed (in accord with the observations on the ease of dehydrohalogenation of $CF_3{\cdot}CH_2{\cdot}CHX_2$ compounds), and that the chlorine atom in $CF_3{\cdot}CCl{:}CH_2$ resists chemical attack.

An alternative route from 2-chloro-3 : 3 : 3-trifluoropropene to trifluoromethylacetylene is by addition of chlorine to give 2 : 2 : 3-trichloro-1 : 1 : 1-trifluoropropane, followed by dehydrochlorination to give the known dichlorotrifluoropropene, $CF_3{\cdot}CCl{:}CHCl$. The acetylene was obtained from this compound in only poor yield on treatment with zinc and alcohol. The

The infra-red spectrum of trifluoromethylacetylene vapour.



isomeric 1-chlorotrifluoropropene could be chlorinated and dehydrochlorinated (route 5*c*) to give 1 : 2-dichloro-3 : 3 : 3-trifluoropropene in much better yield.

The corresponding bromo-compounds were found to give better yields. Treatment of 2 : 3-dibromo-1 : 1 : 1-trifluoropropane with alkali (route 6*a*) gave 2-bromo-3 : 3 : 3-trifluoropropene; some trifluoromethylacetylene was also formed. Further treatment of $CF_3{\cdot}CBr{:}CH_2$ with alkali gave the acetylene in poor yield and the difference in reactivity of the two $C_3H_2BrF_3$ isomers is apparent. Addition of bromine to $CF_3{\cdot}CBr{:}CH_2$ gave the unstable tribromo-compound which, on dehydrobromination, yielded 1 : 2-dibromo-3 : 3 : 3-trifluoropropene. This compound could be debrominated to give trifluoromethylacetylene in almost theoretical yield. Method 6*b* is essentially that used by Henne in his synthesis of the acetylene (private communication). The 1 : 2-dibromotrifluoropropene was also prepared from 1-bromotrifluoropropene by successive bromination and dehydrobromination.

For the preparation of 3 : 3 : 3-trifluoromethylacetylene on a small scale the original method

from trifluoroiodomethane and acetylene is most convenient, whereas for larger-scale preparation the syntheses represented by 6*a* and *b* are preferred.

The infra-red spectrum of trifluoromethylacetylene is shown in the diagram. The C≡C stretching frequency at 4·65 μ. is similar to that in methylacetylene (4·625 μ.; Infra-red absorption spectrogram No. 45 of the American Petroleum Institute, Research Project 44, Nat. Bur. Stand., Washington, D.C.). The acetylenic carbon–hydrogen stretching frequency is at a slightly higher wave-length (3·01 μ.) than in methylacetylene (2·96 μ.). The multiple absorption bands at *ca.* 8·0 and 8·5 μ. are clearly associated with the CF_3 group, and other strong absorption bands can be interpreted in terms of the fundamentals of trifluoromethylacetylene. These results, together with those derived from the potassium bromide region of the spectrum, will be considered in detail in a later communication.

Influence of CF_3 *on Reactivity.*—Since in establishing the formulæ of the compounds described in this paper, no assumptions were made as to the point of attack of halogen atoms, direction of hydrogen halide removal, etc., additional evidence becomes available for the effect of a polyfluoro-group such as CF_3 on nearby hydrogen and halogen atoms. The reactions of three classes of compounds can be distinguished.

(1) When the polyfluoro-group is separated by one or more carbon atoms from the hydrogen or halogen atom under consideration, *e.g.*, $CF_3{\cdot}CH_2{\cdot}\dot{C}H$ or $CF_3{\cdot}CH_2{\cdot}\dot{C}X$, then it has only a slight influence. This is illustrated by the fact that replacement of the hydrogen atom on the CH_2X group in $CF_3{\cdot}CH_2{\cdot}CH_2X$ by chlorine or bromine can occur, although at a slower rate and under more vigorous conditions than for a normal hydrocarbon. Removal of X from $CF_3{\cdot}CH_2{\cdot}CH_2X$ or $CF_3{\cdot}CH{:}CHX$ as HX can readily be achieved. Again, the C–X bond in the $CF_3{\cdot}CH_2{\cdot}CH_2X$ type of compound can undergo Grignard formation and many nucleophilic reactions (unpublished results).

(2) When the hydrogen atom under consideration is attached to the carbon atom adjacent to the polyfluoro-group, then it is substantially protected from attack by atomic reagents, as first observed by Henne and his co-workers in a series of chlorination experiments (Haeckl and Henne, *J. Amer. Chem. Soc.*, 1941, **63**, 2692; Elmore, Hass, Henne, and McBee, *ibid.*, 1940, **62**, 3340; Henne and Whaley, *loc. cit.*; Henne and Hinkamp, *ibid.*, 1945, **67**, 1195). Thus chlorination of $CF_3{\cdot}CH_2{\cdot}CH_2X$ occurs mainly on the terminal carbon atom, and there is a definite tendency at low temperatures for the halogenation to proceed to the $CF_3{\cdot}CH_2{\cdot}CX_3$ stage before attack on the central CH_2 group begins. The "acidic" nature of the hydrogen atoms on the central carbon atom is revealed by their ready removal as HX. A halogen atom on the carbon atom adjacent to the polyfluoro-group, *e.g.*, $CF_3{\cdot}\dot{C}X{\cdot}\dot{C}-$, $CF_3{\cdot}C\dot{X}{:}\dot{C}$, is stabilised, and its removal as HX is difficult, although the removal of halogen from adjacent carbon atoms (*e.g.*, $CF_3{\cdot}CX{:}CHX$) can be effected.

(3) When the hydrogen or halogen atom is attached to the carbon atom carrying the fluorine atom, it becomes resistant to chemical attack, as illustrated by the properties of CHF_3, CF_3Cl, CF_3Br, and CF_3I.

Experimental.

Preparation of Trifluoroiodomethane and 1 : 1 : 1-*Trifluoro*-3-*iodopropane.*—Trifluoroiodomethane was prepared either by the interaction of carbon tetraiodide and iodine pentafluoride or from silver trifluoroacetate and iodine as previously described. The reaction of trifluoroiodomethane with ethylene was carried out in sealed tubes or in a small autoclave; the 1 : 1 adduct formed 80—85% of the product.

Chlorination of 1 : 1 : 1-*Trifluoro*-3-*iodopropane.*—Photochemical chlorination was carried out in a silica tube (200-ml. capacity), cooled by running water and fitted with an efficient reflux condenser cooled to 0°. Chlorine was passed through 1 : 1 : 1-trifluoro-3-iodopropane (11·2 g.) at such a rate that the temperature of the liquid in the flask did not rise above 25°, and that the chlorine was largely absorbed. The tube was irradiated by a Hanovia ultra-violet lamp used without the Wood's filter and situated 24" from the flask. The gases from the condenser were passed through water; when the conditions were carefully controlled, only a trace of organic material was carried through the condenser. When 1·2 mols. of chlorine had been added, the reaction was stopped. A similar experiment in which the fluoroiodopropane in a Pyrex tube was covered by water for the chlorination appeared, on preliminary investigation, to give substantially the same products as in the first experiment. The yields were therefore combined and fractionated to give as main product 3-*chloro*-1 : 1 : 1-*trifluoro*-3-*iodopropane* (11·2 g., 43%) (Found : C, 13·8; H, 1·1; Cl, 13·4; I, 49·2. $C_3H_2ClIF_3$ requires C, 13·9; H, 1·2; Cl, 13·7; I, 49·1%), b. p. 119°, n_D^{20} 1·450. The second product isolated was 3-chloro-1 : 1 : 1-trifluoropropane (2·1 g., 16%) (Found : Cl, 26·5%; *M*, 133. Calc. for $C_3H_4ClF_3$: Cl, 26·8%; *M*, 132·5), b. p. 45—46°, n_D^{25} 1·334. Henne and Whaley, *loc. cit.*, report b. p. 45·1°, n_D^{20} 1·3350, and Henne and Kaye (*J. Amer. Chem. Soc.*, 1950, **72**, 3369) report b. p. 45—46°, n_D^{20} 1·3379, for this compound; its isomer, 2-chloro-1 : 1 : 1-trifluoropropane, has b. p. 30·0°, n_D^{20} 1·3150 (Henne and Whaley, *loc. cit.*).

The third product was also free from iodine and was identified as 3 : 3-dichloro-1 : 1 : 1-trifluoropropane (2·0 g., 12%) (Found : Cl, 41·9%; *M*, 165. Calc. for $C_3H_3Cl_2F_3$: Cl, 42·5%; *M*, 167), b. p. 72—74°, n_D^{25} 1·360. Henne and Whaley (*loc. cit.*) report b. p. 72·4°, n_D^{20} 1·3631. A final product was 3 : 3 : 3-trichloro-1 : 1 : 1-trifluoropropane (2·2 g., 11%) (Found : *M*, 198. Calc. for $C_3H_2Cl_3F_3$: *M*, 201·5), b. p. 93·5—96·5°, n_D^{25} 1·390. Henne and Whaley (*loc. cit.*) report b. p. 95·1°, n_D^{20} 1·3900 for this compound, whose isomer 2 : 3 : 3-trichloro-1 : 1 : 1-trifluoropropane has b. p. 106·8°.

The material between the fractions detailed above amounted to 8% of the product and, although it failed to give any definite product, may have contained small amounts of isomers of some of the above compounds.

When 3-chloro-1 : 1 : 1-trifluoro-3-iodopropane (2·6 g.) was treated with chlorine in a sealed silica tube exposed to ultra-violet radiation, 3 : 3-dichloro-1 : 1 : 1-trifluoropropane (0·9 g., 52%) and 3 : 3-*dichloro*-1 : 1 : 1-*trifluoro*-3-*iodopropane* (0·5 g., 17%) (Found : C, 12·1; H, 0·9. $C_3H_2Cl_2IF_3$ requires C, 12·3; H, 0·7%), b. p. 142—143°, were formed. When this last compound (1·5 g.) was irradiated in a Pyrex tube with chlorine, the only product was 3 : 3 : 3-trichloro-1 : 1 : 1-trifluoropropane, b. p. 94—96°, n_D^{22} 1·389.

Bromination of 1 : 1 : 1-*Trifluoro*-3-*iodopropane*.—Thermal bromination was effected by passing the vapour of 1 : 1 : 1-trifluoro-3-iodopropane (11·2 g.) and a slight excess of bromine through a silica tube of 1-cm. internal diameter and 100-cm. length, heated to 380° over a length of 40 cm. The products were condensed in a trap cooled by liquid air, washed with water to remove bromine, hydrogen bromide, and iodine bromide, dried over phosphoric oxide, and distilled in the presence of a globule of mercury, to yield the following products. (*a*) 3-*Bromo*-1 : 1 : 1-*trifluoro*-3-*iodopropane* (2·1 g., 14%) (Found : C, 12·4; Br, 26·2; I, 41·7. $C_3H_2BrIF_3$ requires C, 11·9; Br, 26·4; I, 41·9%), b. p. 139—140°, n_D^{20} 1·487. (*b*) 3-Bromo-1 : 1 : 1-trifluoropropane (1·2 g., 13%) (Found : Br, 44·9%; *M*, 175. Calc. for $C_3H_4BrF_3$: Br, 45·2%; *M*, 177), b. p. 63°, n_D^{25} 1·358. McBee, Hass, Toland, and Truchan (*loc. cit.*) report b. p. 62—62·5°, n_D^{25} 1·3572. The compound contained no iodine and did not show unsaturation to bromine. (*c*) 3 : 3-Dibromo-1 : 1 : 1-trifluoropropane (3·3 g., 26%) (Found : C, 14·2; H, 0·9%; *M*, 250. Calc. for $C_3H_3Br_2F_3$: C, 14·1; H, 1·1%; *M*, 256), b. p. 110—112°, n_D^{22} 1·426. McBee, Hass, *et al.* (*loc. cit.*) report b. p. 111°, n_D^{25} 1·4242. The fraction was free from iodine and was not unsaturated. (*d*) Intermediate fractions, material formed by decomposition (subsequent experiments revealed the presence of bromotrifluoromethane), and higher-boiling compounds, which were not investigated closely.

Photochemical bromination of 3-bromo-1 : 1 : 1-trifluoro-3-iodopropane (1·50 g.) in a Pyrex tube at room temperature gave 3 : 3-dibromo-1 : 1 : 1-trifluoropropane (0·85 g., 68%) (*M*, 252), b. p. 110—112°.

Samples of 3 : 3-dibromo-1 : 1 : 1-trifluoropropane did not give 3 : 3 : 3-trifluoropropene when refluxed with zinc and absolute alcohol for 5 hours.

Preparation of 1 : 1 : 1-*Trifluoropropane*.—Two methods were used : (*a*) 1 : 1 : 1-Trifluoro-3-iodopropane (22·5 g.) was heated to 280—300° for 24 hours in an autoclave with excess of a saturated hydrocarbon of comparable volatility (*e.g.*, hexane, 50 ml.) and mercury (10 ml.). The 1 : 1 : 1-trifluoropropane (5·0 g., 52%), b. p. −13°, was readily separated from unchanged fluoroiodopropane by distillation. Traces of 3 : 3 : 3-trifluoropropene (2—5%) were removed by mixing the material with chlorine in a tube in the dark for 1 hour, followed by washing of the volatile material with alkali and refractionation. (*b*) 1 : 1 : 1-Trifluoro-3-iodopropane (22·5 g.) was converted into the Grignard reagent which, on hydrolysis, gave 1 : 1 : 1-trifluoropropane in 75% yield.

Chlorination and Bromination of 1 : 1 : 1-*Trifluoropropane*.—*Photochemical chlorination.* Water (5 ml.) was added to a silica flask (500 ml.) which was then filled with 1 : 2 molar amounts of the fluoropropane and chlorine to a pressure of 700 mm. The Hanovia lamp was used 2 feet from the flask which was cooled by water. As chlorination proceeded, further portions of 1 : 1 : 1-trifluoropropane and chlorine were added to keep the pressure near 1 atmosphere, and to prevent the chlorine being present in large excess. The liquid which condensed on the walls of the flask was periodically collected in the ice-cooled and shielded base of the flask by warming of the walls. When a total of 33 g. of the fluoropropane had been treated in this way, chlorination was continued in the liquid phase, using the apparatus described earlier, until the amount of chlorine added and the weight of crude product (54 g.) corresponded to the formation of a dichloro-derivative. Distillation then gave (*a*) 3-chloro-1 : 1 : 1-trifluoropropane (8·2 g., 18%) (*M*, 132), b. p. 44—46°, (*b*) 3 : 3-dichloro-1 : 1 : 1-trifluoropropane (31·6 g., 57%) (*M*, 166), b. p. 72—74°, n_D^{25} 1·359, (*c*) 3 : 3 : 3-trichloro-1 : 1 : 1-trifluoropropane (6·8 g., 10%) (Found : Cl, 52·1%; *M*, 200. Calc. for $C_3H_2Cl_3F_3$: Cl, 52·85%; *M*, 201·5), b. p. 95—95·5°, n_D^{25} 1·388, and (*d*) intermediate fractions and residue (4·2 g.). The composition of the product, monochloro : dichloro : trichloro : intermediate fractions is thus 16 : 62 : 14 : 8 by weight. Under the conditions used, the formation of compounds other than the three indicated above seems doubtful.

Chlorination in the vapour phase was also effected in the absence of water in a 1-l. Pyrex flask at 80—100°. The flask, which had a reflux condenser and a cold finger cooled in ice and shielded from the light, was filled with the fluorohydrocarbon and chlorine (molar ratio 1 : 1) and recharged as necessary to keep the pressure near atmospheric. The chlorinated product from a reaction of this type contained a higher percentage of 2-chloro-1 : 1 : 1-trifluoropropane than formerly. This compound, b. p. 29—31°, was separated by distillation (Found : Cl, 26·0%; *M*, 131. Calc. for $C_3H_4ClF_3$: Cl, 26·8%; *M*, 132·5). The yields of 3-chloro-, 2-chloro-, 3 : 3-dichloro-, and 3 : 3 : 3-trichloro-compounds from 20 g. of reacted trifluoropropane were in the ratios 55 : 18 : 17 : 2 by weight.

The first method of photochemical chlorination was used for most of the work described in this paper.

Thermal chlorination. On passage of equimolar amounts of 1 : 1 : 1-trifluoropropane and chlorine through 6 feet of Pyrex tubing (10-mm. internal diameter), heated to 420°, so as to give a contact time of *ca.* 3 seconds, it was found that 40% of the fluorohydrocarbon (20 g.) underwent reaction to give 3-chloro-, 2-chloro-, 3 : 3-dichloro-, and 3 : 3 : 3-trichloro-1 : 1 : 1-trifluoropropane in yields of 36, 3, 18, and 8% by weight respectively. The 2-chloro-compound (*M*, 131·5) had b. p. 29·5—30·5°, n_D^{25} 1·313, and could be dehydrochlorinated to trifluoropropene as described below.

Thermal bromination. 1 : 1 : 1-Trifluoropropane was treated by the method described earlier. The fluoropropane (33 g.) was passed with excess of bromine through the silica tube heated to 380° and, after preliminary fractionation, unchanged fluorohydrocarbon (10 g.) was recycled. The contact time was very approximately 5 seconds. The combined products were washed with water, dried, and distilled, to yield (*a*) 3-bromo-1 : 1 : 1-trifluoropropane (12·1 g., 20%) (*M*, 176), b. p. 62·5—63·0°, n_D^{25} 1·358, (*b*) 3 : 3-dibromo-1 : 1 : 1-trifluoropropane (26·1 g., 31%) (Found: Br, 61·9%; *M*, 252. Calc. for $C_3H_3Br_2F_3$: Br, 62·5%; *M*, 256), b. p. 110—112°, n_D^{25} 1·425, and (*c*) 2-bromo-1 : 1 : 1-trifluoropropane (2·6 g., 4%) (Found: C, 20·6; H, 2·5; Br, 44·6%; *M*, 177. Calc. for $C_3H_4BrF_3$: C, 20·3; H, 2·3; Br, 45·2%; *M*, 177), b. p. 48—50·5°, n_D^{25} 1·350 [Swarts (*Bull. Soc. chim. Belg.*, 1929, **48**, 106) reports b. p. 49° for this compound]. The amount of (*c*) in the product was much less than observed by McBee *et al.*, *loc. cit.*, however: $CF_3{\cdot}CH_2{\cdot}CH_2Br : CF_3{\cdot}CHBr{\cdot}CH_3 : CF_3{\cdot}CH_2{\cdot}CHBr_2 = 30 : 6 : 64$ and 43 : 25 : 32 by weight respectively.

Chlorination and Bromination of 3-Chloro- and 3-Bromo-1 : 1 : 1-trifluoropropane.—The monochloro- and -bromo-compounds were obtained by chlorination and bromination of 1 : 1 : 1-trifluoro-3-iodopropane and of trifluoropropane as described above, and from trifluoropropylmagnesium iodide (unpublished results). The samples prepared by these routes were identical.

Chlorination of 3-chloro-1 : 1 : 1-trifluoropropane (16·7 g.) was carried out in the liquid phase at 20° in the apparatus described above. Chlorine was added until the weight of crude product corresponded approximately to the formation of the dichloro-derivative, and at such a rate that the liquid remained almost colourless throughout. After washing and drying, distillation gave unchanged monochloro-compound (3·5 g.), 3 : 3-dichloro- (12·3 g., 73%), and 3 : 3 : 3-trichloro-1 : 1 : 1-trifluoropropane (2·5 g., 12%), identified as above. The still residue (1·9 g.) contained a liquid of b. p. higher than that of the trichloro-derivatives and which may have been 2 : 3 : 3-trichloro-1 : 1 : 1-trifluoropropane (b. p. 107°) but was not definitely identified.

Thermal bromination of 3-chloro-1 : 1 : 1-trifluoropropane (6·6 g.) at 400° with a contact time of *ca.* 5 seconds in the apparatus described above gave *3-bromo-3-chloro-1 : 1 : 1-trifluoropropane* (4·3 g., 41%) (Found: C, 16·8; H, 1·4; Br, 37·0; Cl, 17·0%; *M*, 209. $C_3H_3BrClF_3$ requires C, 17·0; H, 1·4; Br, 37·8; Cl, 16·8%; *M*, 211·5), b. p. 91—92°, n_D^{25} 1·395. An unidentified dibromochloro-compound ($CF_3{\cdot}CHBr{\cdot}CHClBr$ or $CF_3{\cdot}CH_2{\cdot}CClBr_2$) was also formed in small amount.

When 3-bromo-3-chloro-1 : 1 : 1-trifluoropropane was added to zinc in refluxing absolute alcohol, trifluoropropene was not formed after 6 hours' heating, whereas the compound could be converted into trifluoromethylacetylene by treatment with potassium hydroxide (see below).

Photochemical chlorination of 3-bromo-1 : 1 : 1-trifluoropropane in the silica apparatus described above also yielded 3-bromo-3-chloro-1 : 1 : 1-trifluoropropane (3·6 g., 51%), b. p. 90·5—91·5°, whose structure was proved as above. A further product isolated was *3-bromo-3 : 3-dichloro-1 : 1 : 1-trifluoropropane* (1·2 g., 14%) (Found: C, 14·4; H, 0·9; Br, 31·8; Cl, 28·8. $C_3H_2BrCl_2F_3$ requires C, 14·6; H, 0·8; Br, 32·5; Cl, 28·9%), b. p. 115—117°. The structure of this compound (1·8 g.) was shown by its dehydrobromination with powdered potassium hydroxide to the known 1 : 1-dichloro-3 : 3 : 3-trifluoropropene (1·0 g., 83%) (*M*, 164), b. p. 53·5—56·0°, n_D^{25} 1·367 (Henne and Whaley, *loc. cit.*, report b. p. 55·1°, n_D^{25} 1·3690), which did not react with zinc and alcohol to give trifluoropropyne. Isomers of $CF_3{\cdot}CH_2{\cdot}CCl_2Br$ (*e.g.*, $CF_3{\cdot}CHCl{\cdot}CHBrCl$) must therefore have been present in only small amounts in the original material.

Thermal bromination of 3-bromo-1 : 1 : 1-trifluoropropane (5·9 g.) at 400° with a contact time of 2—5 seconds gave 3 : 3-dibromo-1 : 1 : 1-trifluoropropane (3·2 g., 37%), b. p. 110·5—111·5°, which did not give trifluoropropene with zinc and ethyl alcohol. A fraction of higher b. p. (112—118°) (0·8 g.) may have contained 2 : 3-dibromo-1 : 1 : 1-trifluoropropane.

Dehydrohalogenation Reactions.—The dehydro-chlorination or -bromination of the compounds described below was effected by adding the compound to a large excess of finely ground, carefully dried, potassium hydroxide which three-quarters filled a 50-, 100-, 250-, or 500-ml. flask (depending on the amount of material). The flask was fitted with a reflux water-condenser and a Dewar condenser which was cooled to −20° for the preparation of 3 : 3 : 3-trifluoropropene or to −45° for the preparation of 3 : 3 : 3-trifluoropropyne, and was connected to a trap cooled by liquid air for the collection of volatile products. The lower one-third of the flask was placed in an oil-bath and the temperature was slowly raised from room temperature to reflux temperature so that the refluxing liquid percolated through potassium hydroxide of gradually increasing temperature before being vaporised. The reactions were carried out at the lowest possible temperature to prevent attack on the trifluoromethyl group.

3-Halogeno-1 : 1 : 1-trifluoropropanes. These readily underwent dehydrohalogenation when the temperature was slowly raised to that necessary for reflux. The iodo-, bromo-, and chloro-compounds gave 3 : 3 : 3-trifluoropropene, b. p. −22°, in 70, 65, and 62% yields respectively on a 20-g. scale.

2-Halogeno-1 : 1 : 1-trifluoropropanes. When heated with powdered potassium hydroxide in a sealed tube at 120—150° for 12 hours, 2-bromo-1 : 1 : 1-trifluoropropane (2·0 g.) gave trifluoropropene (0·4 g., 36%). Similarly, 2-chloro-1 : 1 : 1-trifluoropropane (2·0 g.) gave trifluoropropene (9%) and unchanged chloro-compound (21%) after 24 hours.

The 3 : 3 : 3-trifluoropropene formed in the above reactions was identified by its b. p., molecular weight (96), and infra-red absorption spectrum.

3 : 3-*Dihalogeno*-1 : 1 : 1-*trifluoropropanes*. The first molecule of hydrogen halide was readily removed from the dibromo-compound (13 g.) and subsequent formation of trifluoromethylacetylene was carried out during 8 hours. The contents of the liquid-air trap were then found to contain trifluoromethylacetylene (2·9 g., 62%) and a small amount of 1-*bromo*-3 : 3 : 3-*trifluoropropene* (1·1 g., 12%) (Found : C, 20·6; H, 1·4; Br, 45·0%; *M*, 175. $C_3H_2BrF_3$ requires C, 20·6; H, 1·1; Br, 45·7%; *M*, 175), b. p. 41°.

After 12 hours' refluxing, 3 : 3-dichloro-1 : 1 : 1-trifluoropropane (16·6 g.) similarly yielded trifluoromethylacetylene (4·2 g., 45%) and 1-*chloro*-3 : 3 : 3-*trifluoropropene* (2·1 g., 16%) (Found : Cl, 27·5%; *M*, 129. $C_3H_2ClF_3$ requires Cl, 27·2%; *M*, 130·5), b. p. 21°. Henne and Whaley (*loc. cit.*) state that this dehydrohalogenation can be achieved by alcoholic potassium hydroxide, but give no analytical or physical data for the chlorofluoropropane.

When 3-bromo-3-chloro-1 : 1 : 1-trifluoropropane (2·10 g.) was refluxed over potassium hydroxide for 4 hours, trifluoromethylacetylene (0·52 g., 55%) and 1-chloro-3 : 3 : 3-trifluoropropene (0·1 g., 8%) (*M*, 130) were formed.

3-Chloro-1 : 1 : 1-trifluoro-3-iodopropane similarly yielded the acetylene (0·49 g., 52%) and 1-chloro-3 : 3 : 3-trifluoropropene (0·13 g., 10%) (*M*, 131) after 6 hours. Under the same conditions, 3-bromo-1 : 1 : 1-trifluoro-3-iodopropane (3·00 g.) gave 1-bromo-3 : 3 : 3-trifluoropropene (0·2 g., 11%) (*M*, 173) and trifluoromethylacetylene (0·62 g., 66%).

1-*Chloro and* 1-*bromo*-3 : 3 : 3-*trifluoropropene*. 1-Chlorotrifluoropropene (2·0 g.) gave the acetylene (1·0 g., 70%) after 5 hours, while the bromo-compound (1·3 g.) gave a 72% yield in the same period.

Synthesis of Trifluoromethylacetylene from Trifluoropropene via *Chloro-compounds*.—3 : 3 : 3-Trifluoropropene (14·0 g.) was treated with a slight excess of chlorine in a series of sealed tubes, giving 2 : 3-dichloro-1 : 1 : 1-trifluoropropane (20·1 g., 80%) (Found : C, 22·1; H, 1·7%; *M*, 165. Calc. for $C_3H_3Cl_2F_3$: C, 21·6; H, 1·8%; *M*, 167), b. p. 76·5°, n_D^{25} 1·365. Henne and Whaley report b. p. 76·7°, n_D^{20} 1·3671. In subsequent experiments the olefin and chlorine were mixed in a bulb exposed to daylight; a smooth reaction occurred to give the dichloro-compound in 85% yield.

Treatment of the 2 : 3-dichloro-compound (4·1 g.) with potassium hydroxide for 12 hours gave trifluoromethylacetylene (0·3 g., 12%) and 2-*chloro*-3 : 3 : 3-*trifluoropropene* (1·65 g., 50%) (Found : Cl, 26·9%; *M*, 130. $C_3H_2ClF_3$ requires Cl, 27·2%; *M*, 130·5), b. p. 14—15°. When this compound (2·1 g.) was heated at 130° for 6 hours with powdered potassium hydroxide in a sealed tube, trifluoromethylacetylene was formed in 8% yield. A further experiment in which the chlorofluoropropene was refluxed vigorously at 100-cm. pressure over potassium hydroxide gave the acetylene in 6% yield after 12 hours, with 55% of the chloro-compound (1·1 g.) recovered unchanged.

Treatment of 2-chloro-3 : 3 : 3-trifluoropropene (3·9 g.) with an equivalent amount of chlorine in a sealed tube gave 2 : 2 : 3-*trichloro*-1 : 1 : 1-*trifluoropropane* (5·4 g., 90%) (Found : Cl, 53·1%; *M*, 200. $C_3H_2Cl_3F_3$ requires Cl, 52·9%; *M*, 201·5), b. p. 104—105°, n_D^{25} 1·392. The trichlorofluoropropane (4·8 g.) was refluxed over powdered potassium hydroxide in a flask fitted with a reflux condenser heated to 60° and connected to a trap cooled in liquid air. After 6 hours no further reflux could be observed and the trap was found to contain unchanged trichloro-compound (1·8 g.) and 2 : 3-dichloro-1 : 1 : 1-trifluoropropene (1·2 g., 48%) (Found : C, 21·2; H, 0·8%; *M*, 164. Calc. for $C_3HCl_2F_3$: C, 21·8; H, 0·6%; *M*, 165), b. p. 53—54°, n_D^{25} 1·365. Henne and Whaley (*loc. cit.*) report b. p. 53·7°, n_D^{20} 1·3670.

1 : 2-Dichloro-3 : 3 : 3-trifluoropropene (3·2 g.) was dehalogenated by zinc dust (20 g.) and absolute ethanol (200 ml.) at reflux temperature in a flask fitted with efficient condensers at 15° and −45° and connected to a trap cooled by liquid air. Trifluoromethylacetylene was formed in 15% yield after 40 hours. 40% of the dichloro-compound was recovered unchanged and was re-treated, to give a 25% yield of the acetylene after 120 hours.

1 : 2-Dichloro-3 : 3 : 3-trifluoropropene was also obtained by the addition of chlorine to 1-chloro-3 : 3 : 3-trifluoropropene (2·5 g.), to give 2 : 3 : 3-trichloro-1 : 1 : 1-trifluoropropane (3·4 g., 85%) (Found : *M*, 202. Calc. for $C_3H_2Cl_3F_3$: *M*, 201·5), b. p. 107—107·5°, n_D^{25} 1·398 (Henne and Whaley, *loc. cit.*, report b. p. 106·8°. This compound (3·0 g.) was dehydrochlorinated to the dichlorofluoropropene (2·0 g., 80%). Dehalogenation to the acetylene (18% yield) was effected as described earlier.

Synthesis of Trifluoromethylacetylene from Trifluoropropene via *Bromo-compounds*.—3 : 3 : 3-Trifluoropropene (9·5 g.) was treated with a slight excess of bromine in a series of sealed tubes to give 2 : 3-dibromo-1 : 1 : 1-trifluoropropane (22·1 g., 86%) (Found : C, 14·2%; *M*, 250. Calc. for $C_3H_3Br_2F_3$: C, 14·1%; *M*, 256), b. p. 116°, n_D^{25} 1·427. McBee *et al.* (*loc. cit.*) report b. p. 115·8°, n_D° 1·4281.

The dibromotrifluoropropane (2·6 g.) was readily dehydrobrominated by addition to powdered potassium hydroxide at room temperature and slow increase of the temperature to the reflux point. The reflux condenser was heated to 40°. After 2 hours the volatile products were 2-*bromo*-3 : 3 : 3-*trifluoropropene* (1·1 g., 63%) (Found : C, 20·8; Br, 44·8%; *M*, 175. $C_3H_2BrF_3$ requires C, 20·6; Br, 45·7%; *M*, 175), b. p. 33·5°, n_D^{25} 1·349, and trifluoromethylacetylene (0·04 g., 4%). Further experiments of this type gave yields of 60—65% of the monobromo-compound and 2—8% yields of the acetylene.

The condenser was replaced by two in series at 15° and −40°, respectively. The dibromo-compound (5·2 g.) was slowly heated to reflux with potassium hydroxide, and after 5 hours the volatile products contained 2-bromo-3 : 3 : 3-trifluoropropene (1·6 g., 46%) and the acetylene (0·25 g., 12%).

When 2-bromo-3 : 3 : 3-trifluoropropene (1·7 g.) was vigorously refluxed over potassium hydroxide for 5 hours, a 31% yield of the acetylene was obtained.

Treatment of the 2-bromofluoropropene (5·1 g.) with a slight excess of bromine at 50° in a sealed tube gave 2 : 2 : 3-*tribromo*-1 : 1 : 1-*trifluoropropane* (9·6 g., 98%) (Found : Br, 71·4. $C_3H_3Br_3F_3$ requires Br, 71·6%), b. p. 61°/20 mm., *ca.* 162°/760 mm., n_D^{25} 1·480, as a pale yellow liquid which decomposed appreciably when distilled at atmospheric pressure and slightly when kept at room temperature but could be stored indefinitely at 0°.

The tribromotrifluoropropane (6·7 g.) was dehydrobrominated by addition to powdered potassium hydroxide which had been cooled to −40°. The flask was warmed slowly to a maximum temperature of 140° and a stream of nitrogen passed through it to carry the olefin formed through a condenser kept at 100° and into a trap cooled by liquid air. In this way was obtained 1 : 2-*dibromo*-3 : 3 : 3-*trifluoropropene* (3·2 g., 63%) (Found : C, 14·1; Br, 62·5%; *M*, 253. $C_3HBr_2F_3$ requires C, 14·2; Br, 63·0%; *M*, 254), b. p. 96·5—97·0°, n_D^{25} 1·430. The absence of 1-bromo-3 : 3 : 3-trifluoropropyne, which might result by removal of 2 mols. of hydrogen bromide, was shown by infra-red spectroscopy.

The 1 : 2-dibromo-3 : 3 : 3-trifluoropropene was also prepared from 1-bromo-3 : 3 : 3-trifluoropropene. Addition of bromine to the monobromo-compound (1·75 g.) in a sealed tube gave 2 : 3 : 3-*tribromo*-1 : 1 : 1-*trifluoropropane* (3·2 g., 95%) (Found : Br, 71·0. $C_3H_3Br_3F_3$ requires Br, 71·6%), b. p. 167—168°, 70°/30 mm., n_D^{25} 1·483. Dehydrobromination of this (2·8 g.) was effected as described for its isomer and gave the same olefin, 1 : 2-dibromo-3 : 3 : 3-trifluoropropene (1·6 g., 75%) (*M*, 250), b. p. 95—97°, n_D^{25} 1·431, free from acetylenic compounds.

The 1 : 2-dibromopropene (12·7 g.) was debrominated with zinc dust (20 g.) and absolute ethanol (200 ml.) under a reflux condenser kept at −40°. The reaction was complete after 6 hours. The yield of trifluoromethylacetylene was 4·5 g. (96%)

Purity of Trifluoromethylacetylene.—Samples of the acetylene prepared by the routes described above were tested for purity by boiling-point and molecular-weight determinations, formation of copper and silver derivatives, and examination of the residual gaseous material, and by the infra-red spectrum.

Infra-red Spectrum.—This was measured on a Perkin–Elmer Model 21 Double-beam instrument with a rock-salt prism. Gas pressures in a 5-cm. gas cell with rock-salt windows were measured to 0·5 mm.

UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, April 10th*, 1951.]

NOTES.

551. *Action of N-Bromosuccinimide and N-Bromophthalimide on α-Amino-acids.*

By A. SCHÖNBERG, R. MOUBASHER, and M. Z. BARAKAT.

THERE seem to have been no reports about the reaction mentioned in the title. *N*-Bromosuccinimide reacts vigorously at room temperature with α-amino-acids (*e.g.*, alanine, leucine, and α-phenylglycine), yielding aldehydes containing one carbon atom less, *e.g.*, acetaldehyde, *iso*valeraldehyde, and benzaldehyde respectively. Evolution of carbon dioxide was proved in the case of alanine.

$$\left.\begin{matrix}CH_2{\cdot}CO\\CH_2{\cdot}CO\end{matrix}\right\rangle NBr + R'{\cdot}NH{\cdot}CHR{\cdot}CO_2H \longrightarrow R'{\cdot}NBr{\cdot}CHR{\cdot}CO_2H \text{ (or } R'{\cdot}NH{\cdot}CRBr{\cdot}CO_2H) + \left.\begin{matrix}CH_2{\cdot}CO\\CH_2{\cdot}CO\end{matrix}\right\rangle NH$$

$$\longrightarrow R'{\cdot}N{:}CR{\cdot}CO_2H \xrightarrow{H_2O} CO_2 + NH_2R' + R{\cdot}CHO$$

Sarcosine (*N*-methyglycine) yields formaldehyde when treated with bromosuccinimide. This distinguishes the reaction from the degradation of α-amino-acids effected by polyketones, *e.g.*, benzoquinone (Schönberg, Moubasher, and Mostafa, *J.*, 1948, 176), where no degradation is effected when one of the hydrogen atoms of the α-amino-acid is substituted.

When *N*-bromophthalimide was used to degrade alanine and α-phenylglycine in an aqueous medium, warming was necessary for rapid degradation.

Experimental.—Action of N-bromosuccinimide on α-amino-acids. (*a*) *N*-Bromosuccinimide (1 g.) was allowed to react for 10 minutes without cooling (shaking) with alanine (1·5 g.) or leucine (1·3 g.) in water (35 c.c.), a vigorous reaction taking place with the formation of a red-brown colour. The mixture was then cooled and 2 : 4-dinitrophenylhydrazine hydrochloride in aqueous alcohol was added; an orange precipitate which formed was filtered off and crystallised from alcohol, giving the 2 : 4-dinitro-

phenylhydrazone of acetaldehyde or *iso*valeraldehyde respectively (m. p. and mixed m. p.) in about 50% yield.

When the reaction was carried out with α-phenylglycine, the product was filtered off and to the filtrate 2 : 4-dinitrophenylhydrazine hydrochloride (as above) was added. Benzaldehyde 2 : 4-dinitrophenylhydrazone was obtained, crystallised and identified as above, the yield being about 40%.

(*b*) *N*-Bromosuccinimide (1 g.) and sarcosine hydrochloride (0·6 g.) in water (30 c.c.) were refluxed in the apparatus previously described (Schönberg, Moubasher, and Mostafa, *loc. cit.*) in a stream of carbon dioxide for 90 minutes. The receiver contained 2 : 4-dinitrophenylhydrazine (0·6 g.) in alcohol (20 c.c.). At the end of the experiment concentrated hydrochloric acid (5 c.c.) was added, orange crystals (0·08 g.) being obtained and crystallised from ethyl alcohol. They were proved by their m. p. and mixed m. p. to be formaldehyde 2 : 4-dinitrophenylhydrazone.

Formation of carbon dioxide and succinimide in the degradation. *N*-Bromosuccinimide (1 g., 1 mol.) was added to alanine (1 mol.) in water (35 c.c.) and the reaction allowed to proceed as above; the mixture was then concentrated by heat and brought to dryness under reduced pressure. The residue was recrystallised from benzene and proved to be succinimide by its m. p. and mixed m. p. (yield, 0·25 g.). The evolution of carbon dioxide during the degradation was proved (barium hydroxide).

Action of N-bromophthalimide on α-amino-acids. *N*-Bromophthalimide (freshly prepared; 1 g.) was heated gently under a reflux condenser to about 90° for 3 minutes with alanine (0·4 g.) or α-phenylglycine (0·33 g.) in water (50 c.c.), whereupon a vigorous reaction took place. The mixture was then allowed to cool with continuous shaking and filtered. The solid yielded phthalimide (m. p. and mixed m. p.). The filtrate was treated with excess of 2 : 4-dinitrophenylhydrazine hydrochloride in aqueous alcohol, whereupon orange crystals were formed; the mixture was kept in ice for 1 hour, and the deposit was crystallised from ethyl alcohol and proved to be the 2 : 4-dinitrophenylhydrazone of acetaldehyde and benzaldehyde respectively; the yield was about 25—35%.

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552. *Ethyl Calcioacetoacetate.*

By R. H. HACKMAN.

ETHYL CALCIOACETOACETATE has been prepared previously by the action of metallic calcium (Erdmann and van de Smissen, *Annalen*, 1908, **361**, 66) or calcium carbide (Packendorff, *Ber.*, 1931, **64**, *B*, 948) on ethyl acetoacetate. Only by the first method was the material isolated. It is now shown that it may be conveniently prepared by the action of calcium oxide on the ester. This calcium compound can be used in the typical condensations of ethyl acetoacetate with alkyl and acyl halides, but the yields with it are less than those obtained by use of the sodium derivative. They are, however, comparable with those obtained by the use of calcium ethoxide (Perkin and Pratt, *J.*, 1909, **95**, 159). The calcium derivative thus provides a useful alternative when metallic sodium is not available; moreover, the calcium compound, unlike that of sodium, is not hydrolysed by cold water.

Experimental.—Preparation. (*a*) To ethyl acetoacetate (20 g.) in dry benzene (200 ml.) freshly calcined calcium oxide (6 g.) was added in small portions so as to prevent the reaction becoming too vigorous. The mixture was heated on a steam-bath until the benzene which distilled was no longer turbid. The solid which separated when the solution cooled was collected and exhaustively extracted with hot benzene. The extract, after concentration, was cooled, whereupon the white calcium compound (16·1 g., 85% on ester consumed) separated; it had m. p. 220—221° (Erdmann and van de Smissen, *loc. cit.*, recorded m. p. 215—220°) (Found: Ca, 13·5. Calc. for $C_{12}H_{18}O_4Ca$: Ca, 13·4%).

(*b*) Ethyl acetoacetate (43 g.), dry benzene (170 ml.), and calcium carbide (14 g.) were heated under reflux for 2 hours. The solution was cooled, and the solid which separated was collected and exhaustively extracted with hot benzene. The benzene extract was concentrated and cooled, and the solid which separated was collected by filtration. The derivative (43 g., 87%) had m. p. 220—221°.

Condensation reactions. (*a*) With ethyl iodide. Ethyl calcioacetoacetate (12 g.), dry ethanol (50 ml.), and ethyl iodide (13 g.) were boiled under reflux for 7 hours. During the first 2 hours all the calcium compound went into solution. After removal of the ethanol and excess of ethyl iodide by distillation, water was added, the esters were extracted in ether, this extract was washed with water and dried ($MgSO_4$), the ether evaporated, and the residue distilled, giving ethyl ethylacetoacetate (5·3 g., 42%), b. p. 189°/743 mm.

(*b*) With acetyl chloride. Ethyl calcioacetoacetate (14·3 g.), dry benzene (100 ml.), and acetyl chloride (8·8 g.) were boiled under reflux for 10 hours. After removal of the benzene and excess of acetyl

chloride the product was worked up as in (*a*), above. The diacetoacetic ester (8·7 g., 53%), b. p. 120°/45 mm., was identified as the copper salt, m. p. 148°.

(*c*) With benzoyl chloride. Prepared as for the acetyl derivative, ethyl benzoylacetoacetate (64%) had b. p. 184°/22 mm.

The author acknowledges the assistance given by Professor J. C. Earl, formerly of the University of Sydney, who suggested the work.

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553. *Correction to "Reactions of ω-Substituted Acetophenone Derivatives. Part III."*

By JOHN W. BAKER.

IN the communication named (*J.*, 1932, 2631) it was stated that the reaction of *p*-methoxybenzyl chloride (0·025M.) with pyridine (0·25M.) in 90 wt.-% ethyl alcohol at 30·5° was too rapid for kinetic measurement. Recently, the preparation of *p*-methoxybenzyl chloride for another purpose made it evident that the sample previously used, which was obtained by purification of a purchased sample of "anisyl chloride," could not have been the supposed compound since it was obtained as a solid, m. p. 38°. After so long a period it is not possible to ascertain the exact nature of the material then supplied and, in view of the great increase in our knowledge of the mechanisms of nucleophilic substitutions at saturated carbon which has since accrued, no detailed re-investigation of the kinetics of this reaction has been attempted. A single re-determination, a genuine sample of *p*-methoxybenzyl chloride, b. p. 125°/25 mm. (Found: Cl, 22·7. Calc. for C_8H_9OCl: Cl, 22·7%), being used under the previously prescribed conditions, gave a value of $10^3k_1 \sim 14{\cdot}6$ *in the units used in the earlier communication.* The corresponding value for benzyl chloride was $10^3k_1 = 0{\cdot}093$, so that the effect of a *p*-methoxyl substituent is to increase the velocity about 160-fold. The earlier statement (p. 2633) that this chloride reacts as the ionised form $[p\text{-MeO}{:}C_6H_4{:}CH_2]^+Cl^-$ must thus be replaced by the view that such a structure is probably important in the resonance hybrid structure of the molecule and becomes increasingly so in the transition state of its reaction with pyridine. The contrast with the small *retarding* effect of a *p*-methoxyl substituent in the corresponding reaction of ω-halogenoacetophenones (Baker, *J.*, 1932, 1148) is, however, still valid. No ambiguity applies to the sample of *p*-methoxybenzyl bromide, which was prepared from the corresponding alcohol, obtained from anisaldehyde by a Cannizzaro reaction.

This error emphasises the undesirability of the use of the nomenclature * "anisyl chloride" which is employed by Beilstein for *p*-methoxybenzyl chloride, confusion which is accentuated by the use of the *same* name for *p*-methoxybenzoyl chloride in some chemical catalogues.

THE UNIVERSITY, LEEDS. [*Received, May 17th,* 1951.]

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554. *The Chloro- and Iodo-acetates of α-Amyrin, β-Amyrin, and Lupeol.*

By T. R. AMES, G. S. DAVY, T. G. HALSALL, and E. R. H. JONES.

IN order to obtain pentacyclic triterpene derivatives suitable for *X*-ray examination, the iodoacetates of α-amyrin, β-amyrin, and lupeol have been prepared by treating the corresponding chloroacetates with potassium iodide in acetone (cf. Curtis, *J.*, 1950, 1017). The chloroacetates were obtained from the alcohols with chloroacetyl chloride and diethylaniline in benzene.

Experimental.—All melting points were determined on a Kofler block and are corrected. Rotations were determined in chloroform.

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* Current practice requires the systematic "anisoyl" for the acid radical $MeO{\cdot}C_6H_4{\cdot}CO$. Confusion has also occurred in the past because of the use of "anisyl" for both $MeO{\cdot}C_6H_4{\cdot}CH_2$ and $MeO{\cdot}C_6H_4$; "anisyl" is therefore no longer permitted in the *Journal*, the two radicals being named methoxybenzyl and methoxyphenyl, respectively.—ED.

Lupeol chloroacetate. Lupeol (500 mg.) was dissolved in dry benzene (25 c.c.), and chloroacetyl chloride (1·25 c.c.) and diethylaniline (2·5 c.c.) were added. After 24 hours at 20° the solution was decanted from the separated diethylaniline hydrochloride and washed with water, dilute hydrochloric acid, and again with water. The product obtained after the solution had been dried and the solvent evaporated was adsorbed from *n*-pentane–benzene (1 : 2) on to a column of alumina (50 g.; activity I—II). The fraction (565 mg.) eluted with *n*-pentane–benzene (1 : 1; 200 c.c.) was crystallised from chloroform–methanol, giving platelets of *lupeol chloroacetate* (302 mg.), m. p. 177—184° raised by further recrystallisations from the same solvent to 186—188°, $[\alpha]_D^{20}$ +45° (*c*, 1·30) (Found: C, 76·2; H, 10·25; Cl, 7·35. $C_{32}H_{51}O_2Cl$ requires C, 76·4; H, 10·2; Cl, 7·05%).

α-Amyrin chloroacetate. α-Amyrin (4·0 g.) was treated in like manner yielding *α-amyrin chloroacetate* which was crystallised several times from chloroform–methanol; it formed platelets (1·68 g., 36%), m. p. 200—202°, $[\alpha]_D^{20}$ +73° (*c*, 1·93) (Found: C, 76·45; H, 10·15; Cl, 7·35. $C_{32}H_{51}O_2Cl$ requires C, 76·4; H, 10·2; Cl, 7·05%).

β-Amyrin chloroacetate. β-Amyrin (3·0 g.) gave the *chloroacetate*, which, after being crystallised several times from chloroform–methanol, formed platelets (1·95 g., 55%), m. p. 186·5—188°, $[\alpha]_D^{20}$ +81° (*c*, 1·97) (Found: C, 76·15; H, 10·25; Cl, 6·9. $C_{32}H_{51}O_2Cl$ requires C, 76·4; H, 10·2; Cl, 7·05%).

Lupeol iodoacetate. Lupeol chloroacetate (254 mg.) was heated under reflux with potassium iodide (750 mg.) in acetone (150 c.c.) for 4 hours. The solution was concentrated in a vacuum and the product, isolated by ethereal extraction after dilution with water, was crystallised from chloroform–methanol giving platelets of *lupeol iodoacetate* (266 mg.), m. p. 185—195° raised by two recrystallisations from ethyl acetate–methanol to 195—197°, $[\alpha]_D^{20}$ +37° (*c*, 1·34) (Found: C, 65·0; H, 9·0; I, 21·7. $C_{32}H_{51}O_2I$ requires C, 64·65; H, 8·65; I, 21·35%).

α-Amyrin iodoacetate. The chloroacetate (1·50 g.) yielded the *iodoacetate*, which was crystallised several times from chloroform–methanol and formed flat needles (1·05 g., 59%), m. p. 174—175°, $[\alpha]_D^{20}$ +66° (*c*, 1·95) (Found: C, 64·65; H, 8·4; I, 21·2. $C_{32}H_{51}O_2I$ requires C, 64·65; H, 8·65; I, 21·35%).

β-Amyrin iodoacetate. Prepared from the chloroacetate (1·95 g.) the *iodoacetate* crystallised from chloroform–methanol as platelets (1·25 g.), m. p. 166·5—167°, $[\alpha]_D^{20}$ +74° (*c*, 1·50) (Found: C, 64·8; H, 8·65; I, 21·65. $C_{32}H_{51}O_2I$ requires C, 64·65; H, 8·65; I, 21·35%).

The authors thank Mr. E. S. Morton and Mr. H. Swift for the microanalyses. One of the authors (T. R. A.) thanks the Department of Scientific and Industrial Research for a maintenance grant.

THE UNIVERSITY, MANCHESTER, 13. [*Received, May 24th,* 1951.]

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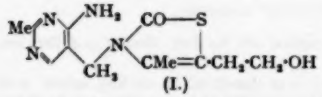
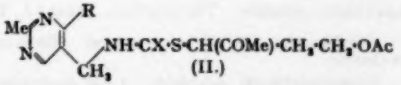
555. *The Synthesis of "Aneurin Thiazolone."*

By PETER SYKES.

IN a recent paper on the mechanism of thiochrome formation from aneurin and aneurin disulphide (Sykes and Todd, *J.*, 1951, 534), much of the argument turned on the structure of an "aneurin thiazolone" obtained by refluxing aneurin disulphide in a number of different solvents. It has now proved possible to confirm that the "thiazolone" has the structure (I) by synthesis and demonstration of the identity of the material with the thiazolone derived from aneurin disulphide.



(I.)



(II.)

It may be prepared either by cyclisation of the intermediate *S*-(3-acetoxy-1-acetylpropyl) *N*-(4-amino-2-methyl-5-pyrimidylmethyl)thiolcarbamate (II; R = NH_2, X = O) in the presence of dilute hydrochloric acid (the ω-acetyl group being simultaneously removed from the side chain), or from 4-amino-5-aminomethyl-2-methylpyrimidine, carbon oxysulphide, and 3-acetoxy-1-chloropropyl methyl ketone, involving treatment with acid (cf. Matsukawa and Iwatsu, *J. Pharm. Soc. Japan*, 1950, **70**, 28). Dilute hydrochloric acid appears to be a fairly general, if somewhat surprising, reagent for this type of closure for, by its aid, we have been able to cyclise 3-acetoxy-1-acetylpropyl *N*-(4-amino-2-methyl-5-pyrimidylmethyl)dithiocarbamate (II; R = NH_2, X = S), 3-acetoxy-1-acetylpropyl *N*-(4-hydroxy-2-methyl-5-pyrimidylmethyl)dithiocarbamate (II; R = OH, X = S), and phenacyl *N*-benzyldithiocarbamate. Too high a temperature (*e.g.*, refluxing) or too prolonged action of the reagent

result in the decomposition of the cyclic compound first formed. With *S*-phenacyl *N*-benzylthiolcarbamate some ring closure could be made to take place, but the starting material underwent extensive hydrolysis.

Experimental.—**3-(4-Amino-2-methyl-5-pyrimidylmethyl)-5-2′-hydroxyethyl-4-methylthiazol-2-one** (" ***aneurin thiazolone*** ") (I): (a) *From the thiolcarbamate* (II; R = NH_2, X = O). *S*-(3-Acetoxy-1-acetylpropyl) *N*-(4-amino-2-methyl-5-pyrimidylmethyl)thiolcarbamate (Sykes and Todd, *loc. cit.*) (0·5 g.) was heated in 3N-hydrochloric acid (10 ml.) on a steam-bath for 10 minutes, cooled, made just alkaline with 3N-sodium hydroxide, and set aside till no more solid separated. The thiazolone crystallised as colourless needles (from ethanol), m. p. and mixed m. p. with natural " aneurin thiazolone," 237° (Found: C, 51·7; H, 6·0; N, 19·7. Calc. for $C_{12}H_{16}O_2N_4S$: C, 51·5; H, 5·8; N, 20·0%). The yield was quantitative.

(b) *From 4-amino-5-aminomethyl-2-methylpyrimidine.* 4-Amino-5-aminomethyl-2-methylpyrimidine dihydrochloride (2·1 g.) was treated with a solution of potassium hydroxide (1·1 g.) in methanol (25 ml.), the separated potassium chloride was filtered off, and 3-acetoxy-1-chloropropyl methyl ketone (1·8 g.) added, followed by 20% aqueous ammonia (1 ml.) and an ice-cold saturated solution of carbon oxysulphide in methanol (100 ml.). A stream of dry carbon oxysulphide was passed in for 10 minutes, and the solution then evaporated to dryness *in vacuo*. The residue was washed with water, taken up in hydrochloric acid (10%; 25 ml.), warmed on the steam-bath for 10 minutes, and finally neutralised with 10% sodium hydroxide solution. The product separated as colourless needles (from ethanol), m. p. and mixed m. p. with natural " aneurin thiazolone," 237°.

UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, June 4th*, 1951.]

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556. *Acenaphthene Series. Part VI.* The Three Nuclear-substituted Ethylacenaphthenes.*

By ERIC ILLINGWORTH and ARNOLD T. PETERS.

THE liquid ethylacenaphthene, prepared by the Friedel–Crafts reaction from acenaphthene by means of ethyl bromide and aluminium chloride, was shown by Nürsten and Peters (*J.*, 1950, 2389) to be 2-ethylacenaphthene, whereas solid 3-ethylacenaphthene was obtained by reducing 3-acetylacenaphthene. 1-Ethylacenaphthene has been recorded as both an oil and a solid, and since none of its derivatives has been prepared it was synthesised and its derivatives compared with the isomers obtained from 2- and 3-ethylacenaphthene, respectively.

1-Chloromethyl-2-ethylnaphthalene, recorded as an oil by Buu-Hoï and Cagniant (*Rev. Sci.*, 1942, **80**, 271) and by Bachmann and Sheehan (*J. Amer. Chem. Soc.*, 1941, **63**, 2599), was isolated by the present authors in colourless plates, m. p. 69—70°, and converted by a modification of the published methods (*locc. cit.*) into 1-ethylacenaphthenone, and thence into 1-ethylacenaphthene, m. p. 29—30° (picrate, m. p. 104—105°). Oxidation then gave 2-ethylnaphthalic anhydride, which afforded an imide and a *N*-methylimide which differed from the isomers obtained similarly from 3- and 4-ethylnaphthalic anhydride. Moreover, the scarlet vat dye prepared from 1-ethylacenaphthenequinone by condensation with 2-hydroxythionaphthen, differed from the analogous thioindigoid dyes obtained from 2- and 3-ethylacenaphthenequinone. The previous Table (*J.*, 1950, p. 2389) is now complete.

Experimental.—M. p.s are corrected. Micro-analyses were carried out by Drs. Weiler and Strauss, of Oxford.

2-Ethylnaphthalic anhydride. 1-Ethylacenaphthenone (3 g.) was dissolved in boiling acetic acid (140 c.c.), the flame removed, and powdered sodium dichromate (12 g.) added at such a rate as to keep the mixture boiling gently. The mixture was then refluxed for 3 hours and added to water, the precipitate was collected and extracted with boiling 5% aqueous sodium carbonate, and the cooled extracts were acidified. 2-*Ethylnaphthalic anhydride* crystallised from alcohol in colourless needles, m. p. 165—166° (2·5 g., 73%) (Found: C, 73·9; H, 4·2. $C_{14}H_{10}O_3$ requires C, 74·3; H, 4·4%). On admixture with 3-, m. p. 192—193°, and 4-ethylnaphthalic anhydride, m. p. 164—164·5°, it melted at 140—150° and 128—132°, respectively.

2-Ethylnaphthalimide. The anhydride (1 g.), alcohol (4 c.c.), and aqueous ammonia (*d* 0·88; 4 c.c.) were heated at 100° for 4 hours in a sealed tube. The *imide* crystallised from alcohol in prismatic needles, m. p. 221·5—222·5° (Found: C, 74·4; H, 4·9; N, 6·1. $C_{14}H_{11}O_2N$ requires C, 74·3; H, 5·1; N, 6·2%). It depressed the m. p. of the 3-, m. p. 217—218·5°, and 4-isomer, m. p. 195—196°, to 180—186° and 175—180°, respectively.

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* Part V, *J.*, 1951, 1602.

2-Ethyl-N-methylnaphthalimide. The anhydride (1 g.), 33% aqueous methylamine (4 c.c.), and alcohol (4 c.c.) at 100° (1 hour) gave the *N-methylimide*, which crystallised from alcohol in prismatic needles, m. p. 117—118° (Found: C, 75·5; H, 5·5; N, 6·0. $C_{13}H_{13}O_2N$ requires C, 75·3; H, 5·4; N, 5·9%). Admixture with the 3-, m. p. 113—114°, and 4-isomer, m. p. 140°, gave m. p.s 84—95° and 85—97° respectively.

1'-Ethyl-1-thionaphthen-7'(or *-6'*)*-acenaphthenylindigo.*—1-Ethylacenaphthenequinone (1 g.) in boiling acetic acid (20 c.c.) was mixed with 2-hydroxythionaphthen (2 g.) in acetic and hydrochloric acids (5 : 1; 20 c.c.); during several minutes' boiling, a red precipitate was formed progressively. Crystallisation of this from toluene gave bright scarlet prismatic needles, m. p. 206·5—207°, of the *dye* (Found: C, 77·2; H, 4·1; S, 9·4. $C_{32}H_{14}O_2S$ requires C, 76·9; H, 4·1; S, 9·4%), which dissolved in concentrated sulphuric acid with a green colour, becoming pink on dilution. Cotton is dyed salmon-pink from a violet alkaline dithionite (hydrosulphite) vat. On admixture with the 2'(or 5')-, m. p. 204—206°, or the 3'(or 4')-isomeric dye, m. p. 216—217°, the m. p. was depressed to 170—185° and 174—190°, respectively.

CLOTHWORKERS' RESEARCH LABORATORY,
THE UNIVERSITY, LEEDS.

[*Received, June 7th*, 1951.]

OBITUARY NOTICE.

ISIDORE ELKANAH BALABAN.

1896—1951.

ISIDORE ELKANAH BALABAN was born in the Midlands in 1896 and was keenly interested in Science from an early age; nevertheless, he volunteered for the army during the first World War, and served with distinction, first in the Manchester Regiment and later in the Gas Corps. He was commissioned and was awarded the D.C.M.

He returned to the College of Technology, Manchester, after the war and, having graduated, became Professor Pyman's research assistant and took the M.Sc. and Ph.D. degrees. It was at this time that he became interested in glyoxalines, and a number of papers were published in this and in related fields under the inspiration of his chief. He then obtained an appointment with the Medical Research Council under Sir Henry Dale and in 1926 he joined the research staff of Messrs. May and Baker Ltd. At this time, their research staff was being organised under Dr. A. J. Ewins and the work then was mainly on organic arsenicals; Balaban made his own important and distinctly individual contribution to the organic chemistry of arsenic and of antimony. From 1925 to about 1932, very few of his papers were published with other authors but his work indicated a very high degree of skill and ingenuity and more than a trace of the influence of Pyman and of the Medical Research Council training.

Balaban was not, at first acquaintance, the easiest of collaborators but, after the ice had been broken, he proved to be a very reliable source of knowledge, experience, and new ideas and to be a very original thinker in his own specialised fields.

When this Company was re-organised after its transfer to Dagenham, he left to join Imperial Chemical Industries Limited at Blackley, thus returning to his native city. He gained his D.Sc. degree in 1935 and joined the Geigy Company Ltd. in 1941. His progress there resulted in due course in his appointment as Head of the Pharmaceutical Department; his interests in chemotherapy and the synthesis of chemicals for use in medicine continued and he was responsible for a number of patents and scientific publications with the Geigy Company.

His tragic death in a railway accident in Manchester in May, 1951, was a great shock to all who had known him and deprived science of a very able and conscientious worker.

He had just been elected to the Committee of the Fine Chemicals Group of the Society of Chemical Industry. It was typical of Balaban that his military career and distinctions were unknown to the writer until after his death. He had a great love of Nature and studied with keen enjoyment, not only his main interest, organic chemistry, but many of its manifestations such as horticulture and ornithology. His enthusiasm and honesty of purpose were outstanding and will be long remembered by his friends and colleagues.

M. A. PHILLIPS.

THE CHEMICAL SOCIETY

SCIENTIFIC MEETINGS DURING OCTOBER, 1951

LONDON.

Thursday, October 18th, 1951, at 7.30 p.m.

Tilden Lecture, **The Contributions of Wave Mechanics to Chemistry,** by Professor C. A. Coulson, D.Sc., F.R.S.

ABERDEEN.

Thursday, October 25th, 1951, at 7.30 p.m.

Lecture, **Applications of Electric Dipole Moments to the Determination of Structure,** by Dr. L. E. Sutton, M.A., F.R.S.

Joint meeting with the Royal Institute of Chemistry and the Society of Chemical Industry, to be held in the Chemistry Department, Marischal College, Aberdeen.

BIRMINGHAM.

Friday, October 26th, 1951, at 4.30 p.m.

Lecture, **Some Aspects of Oxidation and Corrosion,** by Dr. U. R. Evans, M.A., F.R.S.

Joint meeting with the University Chemical Society to be held in the Chemistry Lecture Theatre, The University, Edgbaston, Birmingham.

GLASGOW.

Friday, October 26th, 1951, at 3.30 p.m.

Lecture, **Chemicals as a Source of Energy, with Special Reference to Industrial Applications and to Jet Propulsion,** by Dr. J. Taylor, M.B.E., D.Sc., F.R.I.C.

Joint meeting with Alchemists' Club and Andersonian Chemical Society to be held in the Chemistry Department, The University, Glasgow.

HULL.

Thursday, October 25th, 1951, at 6 p.m.

Lecture, **Some Methods of Measuring Reaction Velocities in Solution,** by Mr. R. P. Bell, M.A., F.R.S.

To be given in the Science Lecture Theatre, University College, Hull.

LIVERPOOL.

Thursday, October 25th, 1951, at 4.30 p.m.

Lecture, **Some Recent Work on Large Ring Compounds,** by Professor Wilson Baker, D.Sc., M.A., F.R.S.

Joint meeting with the Royal Institute of Chemistry, the Society of Chemical Industry, and the British Association of Chemists to be held in the Chemistry Lecture Theatre, The University, Liverpool.

MANCHESTER.

Thursday, October 18th, 1951, at 6.30 p.m.

Lecture, **Some Problems in the Oxidation of Hydrocarbons,** by Professor A. R. Ubbelohde, M.A., D.Sc., F.R.S.

Joint meeting with the Royal Institute of Chemistry and the Society of Chemical Industry to be held in the University, Manchester.

NEWCASTLE AND DURHAM.

Friday, October 26th, 1951, at 5.30 p.m.

Bedson Club Lecture, **Recent Advances in the Chemistry of Insulin,** by Professor A. C. Chibnall, M.A., Sc.D., F.R.S.

To be given in the Chemistry Building, King's College, Newcastle-on-Tyne. All Fellows are invited.

NORTHERN IRELAND.

Wednesday, October 17th, 1951, at 7.15 p.m.

Lecture, **Microgram Analysis ; Past, Present, and Future,** by Dr. C. L. Wilson, M.Sc., F.R.I.C.

Joint meeting with the Royal Institute of Chemistry and the Society of Chemical Industry, to be held in the Lecture Theatre, Department of Agricultural Chemistry, Queen's University, Belfast.

OXFORD.

Monday, October 22nd, 1951, at 8.15 p.m.

Alembic Club Lecture, **Some Equilibria and Reactions of Sulphur,** by Dr. G. Gee, M.Sc., F.R.S.

To be given in the Physical Chemistry Laboratory, South Parks Road, Oxford. All Fellows are invited.

ST. ANDREWS AND DUNDEE.

Friday, October 19th, 1951, at 5.15 p.m.

Lecture, **William Davidson, "Nobilis Scotus" : a Romance of 17th-Century Chemistry,** by Professor John Read, M.A., Sc.D., F.R.S.

Joint meeting with St. Andrews University Chemical Society to be held in the Chemistry Department, United College, St. Andrews.

SHEFFIELD.

Thursday, October 11th, 1951, at 7.15 p.m.

Lecture, **Colour Photography,** by Dr. H. Baines, F.R.I.C.

Joint meeting with the Royal Institute of Chemistry to be held in the Chemistry Lecture Theatre, The University, Sheffield.

SOUTHAMPTON.

Tuesday, October 30th, 1951, at 7 p.m.

Lecture, **Nitration and Nitrating Agents,** by Professor C. K. Ingold, D.Sc., F.R.S.

Joint meeting with the Portsmouth and District Chemical Society to be held in the Municipal College, Portsmouth.

SOUTH WALES.

Friday, October 26th, 1951, at 5.30 p.m.

Lecture, **Electrochemical Aspects of Colloid Science,** by Mr. D. C. Henry, M.A.

Joint meeting with the Royal Institute of Chemistry to be held in the Chemistry Department, University College, Swansea.

PROCEEDINGS

OF THE

CHEMICAL SOCIETY

OFFICIAL ANNOUNCEMENTS

DEATHS.

The Council regret to announce the deaths of the following Fellows:

	Elected.	Died.
Arthur John Allmand (N.W.11) (C. 1920—23, 1932—34)	May 7th, 1908.	July 4th, 1951.
Arthur James Curtin Cosbie (Manchester)	Mar. 19th, 1942.	May 20th, 1951.
Thomas Harold Fairbrother (S.E.3)	June 15th, 1944.	Aug. 4th, 1951.
Robert Graham (Edinburgh)	June 16th, 1938.	Oct. 14th, 1950.
Alexander McKenzie (Barnhill)	May 2nd, 1901.	June 11th, 1951.
Samuel Morris (Lane Cove, Australia)	Apr. 16th, 1942.	May 13th, 1951.
Andrew Orr (N.21)	Mar. 16th, 1944.	July 25th, 1951.
Walter James Powell (S.E.18)	Aug. 17th, 1944.	May 25th, 1951.
William Raitt (Dorking)	Feb. 15th, 1912.	Dec. 10th, 1950.
David Ruxton (E.C.2)	Feb. 16th, 1950.	May 24th, 1951.
Ernest Samuel Spencer (Surbiton)	Feb. 1st, 1883.	July 5th, 1951.
Joseph George Weighall (Olton)	Sept. 17th, 1942.	Feb. 20th, 1951.
Walter Collingwood Williams (Liverpool)	Mar. 5th, 1885.	Apr. 4th, 1951.

ELECTION OF NEW FELLOWS.

Honorary Fellowship—Professor A. B. Lamb.

The President formally conferred Honorary Fellowship of the Society on Professor Arthur Beckett Lamb at a ceremony held on September 3rd, 1951, at the Manhattan Centre, New York, during the Seventy-fifth Anniversary Celebrations of the American Chemical Society.

Fellows.

The following 46 candidates were elected Fellows of the Society on July 16th, 1951:

Joseph E. P. Apellaniz.
Doreen Mary Bovey.
Mrs. Helena Bradbury.
Phillip Emerson Braid.
William Burns Brown.
Peter Martin Burrill.
Albert Smith Carter.
John Pruen Cordner.
Joseph Bernard Cotton.
Basil Anderson Dunell.
Oliver John Elphick.
William Spencer Fones.
Raymond Eric Ford.
Erling Hagerup.
Raymond John Harrisson.
Irwin Charles Haye.
John Hudson.
Arthur King Ingberman.
Edward Alexandre Johnson.
Richard Lee Kenyon.
Richard David Langford.
Aaron Levy.
William Lonie.
William Arthur Evelyn McBryde.
John Rowan McCallum.
Norman Macleod.
Sidney Benjamin Maerov.
Matthew William Miller.
John Wilson Ogleby.
Ernest Walter Peacock.
Richard Dennistoun Ray.
Carleton Whitman Roberts.
Kenneth Thomas Seal.
Albert Herman Sherman.
Vernon Jack Shiner, jun.
Reginald Slater.
Paul Crosby Stanley.
William Alan Sweeney.
Johannes Tiedt.
Norman Gordon Maxwell Tuck.
Derek George Turpin.
Eric Wait.
William Jack Wasserman.
Kenneth Whittaker.
Thomas Ffrancon Williams.
James Crawford Woodward.

and the following 48 on August 16th, 1951 :

William Peter James Baily.
Cyril Raymond Barnes.
Gail Hubert Birum.
Bernard George Boggiano.
George Buncher.
John Parkman Butler.
Michael John Cabell.
Derek Carr.
Germaine Cauquil.
Peter Bernard Checkland.
John Garfield Chenoweth.
Stefano Chiavarelli.
Kenneth William Chilvers.
Richard Henry Cousens.
George Cameron Davidson.
Peter Glendinning Dunley.
Howard E. Everson.
John Thomas Fallows.
Rodney Ham Foss.
Robert Herrmann Franke.
Maurice Hilary George.
Frederick William Gray.
Michael William Hill.
Donald Raynor Hogg.
Eric Illingworth.
Karl Heinz Willi Imhausen
Henrietta Irwin.
Emmanuel St. Clair Fitzgerald Jones.
Denis Anthony Kinsley.
Jay Kazuo Kochi.
George Frederick Laws.
Alexander Lecker.
George Wilson Longstaff.
V. Harold Maddox, jun.
Jerome L. Martin.
Anton Mudrak.
Harry Erwin Nürsten.
Albert Stanford Orr.
Douglas Roy Penman.
Quentin Richard Petersen.
Jack Reynolds Plimmer.
Edmond Penrod Purser.
Jan Hynek Seidler.
John Frederick Smith.
Norman Fletcher Taylor.
John Michael Turner.
Saad Wasif.
Desmond Michael Sherlock Wheeler.

GIFTS OF PUBLICATIONS.

Acknowledgment is made of gifts of the Society's publications from Mr. F. O. Isaac and Dr. G. Whittingham.

RESEARCH FUND.

A meeting of the Research Fund Committee will be held in November next. All persons who have received grants, and whose accounts have not been declared closed by the Council, are informed that reports must be received by the Society not later than November 1st, 1951.

Applications for grants, to be made on forms obtainable from the General Secretary, must be received on or before November 1st, 1951. Applications from Fellows will receive prior consideration.

Attention is drawn to the fact that the income arising from the donation of the Worshipful Company of Goldsmiths is principally devoted to the encouragement of research in inorganic and metallurgical chemistry, and that the income from the Perkin Memorial Fund is to be applied to investigations relating to problems connected with the coal-tar and allied industries.

NOMENCLATURE SUB-COMMITTEES.

Chemical nomenclature used in the Society's publications is decided by the Publication Committee, the Editors being considered as expert advisers and acting as executive officers of the Committee in day-to-day administration of the nomenclature policy. When major decisions on nomenclature require to be taken the Publication Committee appoints Sub-Committees to study the problems and tender advice; these are *ad hoc* Sub-Committees which, having reported, are then disbanded. There are no Standing Committees, and the life of a Sub-Committee varies greatly according to the nature of the questions under consideration.

All questions referring to nomenclature or to the Sub-Committees should be addressed to the Editor.

The following list gives some recent Sub-Committees, those marked * being still active :

Order of Prefixes. Professor C. S. Gibson, Dr. G. M. Bennett, Dr. F. Bergel, Professor C. K. Ingold, The Editors. Appointed, November 11th, 1948. Disbanded, February 16th, 1950. The Sub-Committee's report in favour of the alphabetical order of prefixes for organic nomenclature was adopted (cf. *J.*, 1950, 3699).

Inorganic Nomenclature, to consider the Ewens-Bassett proposals. Dr. G. M. Bennett, Dr. E. J. Bowen, Professor H. J. Emeléus, Professor J. R. Partington, the Editors. Appointed, March 17th, 1949. Disbanded, June 2nd, 1949. The Sub-Committee advised that these proposals (*Chem. and Ind.*, 1949, 131), with minor modifications, should be supported at the meeting of the I.U.P.A.C. Commission on Nomenclature of Inorganic Chemistry in September 1949, and the British members of the Commission were informed accordingly.

**Carbohydrate Nomenclature.* Professor S. Peat (Chairman), Professor W. Baker, Professor H. Burton, Professor E. L. Hirst, Dr. J. Honeyman, Professor C. K. Ingold, Professor W. H. Linnell, Dr. B. Lythgoe, Dr. E. G. V. Percival, Professor M. Stacey, Professor A. R. Todd, the Editors. Representatives of the Biochemical Society: Dr. D. J. Bell, Dr. W. Klyne, and Dr. T. S. Work. Appointed, October 20th, 1949. This Sub-Committee is reconsidering the British nomenclature of carbohydrates and is in active consultation with the Standing Committee on Nomenclature of the Division of Sugar Chemistry of the American Chemical Society with a view to reconciling British and American practice. Progress has already been made and personal exchange of views is envisaged in New York in September 1951.

I.U.P.A.C. 1949 *Organic Nomenclature Report.* Dr. G. M. Bennett, Professor H. Burton, Dr. L. N. Owen, Dr. J. Walker, the Editors. Appointed, October 12th, 1950. Disbanded, December 14th, 1950. This Sub-Committee examined the 1949 Report of the I.U.P.A.C. Commission on Nomenclature of Organic Chemistry (*Compt. rend. 15th Conf. Amsterdam*, 1949, p. 127). The Publication Committee accepted its advice that the recommendations of the Commission be adopted in the Society's publications, except that the Commission should be asked to reconsider four specific points. Most of the recommendations should be brought into force at once, but certain major changes should await reprinting of the I.U.P.A.C. Report in our *Journal*; this reprint will embody such alterations as are made at the 1951 meeting of the Commission, together with comments to be compiled jointly by the Editor and Dr. A. M. Patterson.

**Organo-phosphorus Nomenclature.* Professor A. R. Todd (Chairman), Dr. F. Bergel, Professor E. D. Hughes, Dr. F. G. Mann, Dr. H. N. Rydon, Dr. B. C. Saunders, Mr. L. T. D. Williams, the Editors. Appointed, December 14th, 1950. This Sub-Committee is engaged in reconsideration of the nomenclature of organic compounds of phosphorus (and of related compounds) with a view to (i) reconciling British and American practice and (ii) submission of revised proposals to I.U.P.A.C. Active collaboration is in progress with the American Chemical Society's Advisory Committee on Organophosphorus Nomenclature, and personal consultations have been arranged to take place in New York in 1951.

I.U.P.A.C. Inorganic Nomenclature, Draft Report 1951. Dr. H. Bassett, Dr. G. M. Bennett, Dr. E. J. Bowen, Dr. G. H. Cheesman, Professor H. J. Emeléus, Dr. F. Fairbrother, Dr. R. S. Nyholm, Professor J. R. Partington, the Editors. Appointed, February, 15th, 1951. Disbanded, May 10th, 1951. The British members of the I.U.P.A.C. Commission of Nomenclature of Inorganic Chemistry had asked for advice on proposals to come before the Commission in September 1951. The Sub-Committee examined the proposals, and its comments were approved by the Publications Committee.

In addition to the above the Publication Committee has arranged, in May 1951, a Joint Committee* with other interested Societies and Institutions to consider the reproduction and availability of infra-red absorption spectra. Members of this Joint Committee are: Chemical Society: Professor E. D. Hughes, Dr. A. E. Martin, Dr. R. S. Cahn. Royal Society and Chemical Society: Dr. H. W. Thompson (Chairman). Physical Society: Dr. A. C. Menzies. Faraday Society: Dr. N. Sheppard. Institute of Petroleum: Dr. H. Powell. It is hoped to exchange views with other Societies and Institutions both in this country and overseas.

BRITISH-AMERICAN COLLABORATION ON NOMENCLATURE.

With recurrence of more normal scientific conditions after the war, consultation between our Society's Editors and the staff of *Chemical Abstracts* has been resumed on a continually increasing scale (cf. *J.*, 1950, 3705). There is, too, growing collaboration

between Nomenclature Committees of the American Chemical Society and of our Society. It is considered that adherence, so far as possible, to international decisions is most desirable (cf. *Proc.*, 1951, 21) and that British–American agreement on broad policy is an essential preliminary to international agreement. The Publication Committee of the Chemical Society and the Board of Directors of the American Chemical Society have therefore agreed to consult with each other before major changes in nomenclature are made, thus giving formal sanction to the practice of informal consultation.

The Editor is responsible for organising the British participation in both the international and the American consultations, and all questions should be referred to him in the first place.

INTERNATIONAL CONGRESS ON ANALYTICAL CHEMISTRY, 1952.

The Congress will meet at Oxford during the period September 4th—9th, and the scientific sessions will be held in the rooms of the " Examinations Schools."

Three main Congress Lectures have been arranged. The programme for the scientific sessions is in the hands of a Programme Committee, under the Chairmanship of Dr. G. M. Bennett, C.B.E., F.R.S., the Government Chemist, with Mr. L. W. Codd, M.A., of Imperial Chemical Industries, Ltd., as Honorary Secretary. This Committee has appointed a number of " Advisers " who are expert in their particular fields, and the draft programme has been divided on a basis of broad techniques.

Papers will be issued in pre-print form before the meeting, and the contributors will give only a brief summary of their papers, most of the time being given over to discussion. Arrangements have been made to publish the whole of the Proceedings in a special number, or numbers, of the *Analyst*, as soon as possible after the Congress.

During the period of the Congress it is proposed to have in operation working demonstrations illustrating new techniques or special applications of older techniques in analytical chemistry.

In addition to this, and quite separate from it, there will be a trade exhibition, comprising apparatus and books, under the management of Mr. W. Thompson, of Imperial College.

Some visits have been planned and, at the week-end, a number of excursions to places of interest will be arranged.

The arrangements at Oxford, for the time being, are in the hands of Dr. F. M. Brewer. Sir Wallace Akers, C.B.E., is Chairman of the Finance Committee, and the Honorary Secretary of the Congress is Mr. R. C. Chirnside, F.R.I.C., Research Laboratories, The General Electric Co., Ltd., Wembley, England.

MEETINGS OUTSIDE LONDON

BIRMINGHAM.

" Recent Developments and Applications of Infra-red Spectroscopy," by Dr. H. W. Thompson, F.R.S.

A joint meeting with the University Chemical Society was held in the Chemistry Lecture Theatre of the University, Edgbaston, on April 26th, 1951, with Dr. L. L. Bircumshaw in the Chair.

Dr. Thompson reviewed the whole field of infra-red spectroscopy and dwelt particularly on the recent work carried out at the University of Oxford, including the use of gratings for very high resolution, low-temperature measurements, the use of polarised radiation for crystal structure determination, and the measurement of absolute intensities of absorption.

At the conclusion, a vote of thanks to Dr. Thompson, proposed by Dr. D. H. Whiffen, was carried enthusiastically.

LIST OF APPLICATIONS FOR FELLOWSHIP

(Fellows wishing to lodge objection to the election of these candidates should communicate with the Honorary Secretaries within ten days of the date of publication of the Journal *for September, 1951. Such objections will be treated as confidential. The forms of application are available in the Library.)*

***Ammar, Ibrahim Ali,** B.Sc. (Cairo). Egyptian. Royal College of Science, Imperial Institute Road, S.W.7. Research Student. *Signed by:* R. G. H. Watson, D. F. Parsons, J. T. Law.

***Barlin, Gordon Bruce,** B.Sc. (Sydney). British. Lansdowne, Manning River, N.S.W., Australia. Research Student, University of Sydney. *Signed by:* R. J. W. Le Fèvre, J. Cymerman, T. Iredale.

***Bartels-Keith, James Richard,** B.A. (Cantab). British. Flat 4, 196, Cromwell Road, S.W.5. Research Student. *Signed by:* H. G. Khorana, J. G. Buchanan, P. Sykes.

***Bauer, Henry Hermann,** B.Sc. (Sydney). British. 4, Bayview Street, Bronte, Sydney, N.S.W., Australia. Student. *Signed by:* R. J. W. Le Fèvre, T. Iredale, H. C. Freeman.

Bellamy, Lionel John, B.Sc. and Ph.D. (Lond.). British. The Cottage, 116, Widmore Road, Bromley. Chemist, Ministry of Supply. *Signed by:* J. D. Strickland, E. W. R. Press, R. G. Monk.

Blackman, Edward John, B.Sc. (Bristol). British. 505, Southmead Road, Westbury-on-Trym, Bristol. Research Chemist, Monsanto Chemicals Ltd. *Signed by:* A. M. Spivey, K. C. Bryant, B. B. Millward.

***Bowe, William Harold,** B.Sc. (Sheffield). British. 74, Ashdown Avenue, Scunthorpe. Organic Research Chemist. *Signed by:* A. H. Lamberton, J. Barrott, R. E. Davies.

***Broadhurst, Thomas,** B.Sc. (L'pool). British. 3, St. Clair Drive, Southport. Research Student, Liverpool University. *Signed by:* W. B. Whalley, R. J. S. Beer, D. H. Johnson.

Bull, Kenneth Bateson. British. 78, Fairway Avenue, Kingsbury, N.W.9. Research Assistant, Eugene, Ltd., N.W.9. *Signed by:* E. W. G. Jackman, G. T. Walker, F. N. Harwood.

***Burwell, Barbara Sybil,** B.Sc. (Bristol). British. 30d, Collingham Place, Earls Court, S.W.5. Chemist, J. Lyons & Co., Ltd., W.14. *Signed by:* J. H. Bushill, M. A. Birch, G. E. Forstner.

***Busby, Arthur,** B.Sc. (Lond.). British. 80, Mansell Road, Greenford. Scientific Officer, Glaxo Laboratories, Ltd., Greenford. *Signed by:* F. F. Stephens, J. Elks, J. Attenburrow.

Cooper, Thomas Peter, Ph.C. British. 44, Ophir Road, North End, Portsmouth. Deputy Chief Pharmacist, St. Mary's Hospital, Portsmouth. *Signed by:* K. C. Aylett, J. A. Durant, J. A. Stock.

Doban, Robert Charles, B.S. (Yale). American. 10, Tilton Terrace, Madison, Wisconsin, U.S.A. Graduate Student, University of Wisconsin. *Signed by:* A. L. Wilds, W. S. Johnson, S. M. McElvain.

***Embrey, Peter Godwin,** B.A. (Oxon.). British. Rough Close Farm, Rough Close, Stoke-on-Trent. Research Student. *Signed by:* D. Ll. Hammick, F. M. Brewer, H. Irving.

Farnworth, Thomas Richard. British. 27B, Grove Park, Liverpool, 8. Analytical Chemist. *Signed by:* J. F. Clark, N. Heron, H. Weatherall.

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"The influence of configuration on the thermal stability of *N*-menthylphthalamic acids." By A. KILLEN MACBETH, J. A. MILLS, and W. G. P. ROBERTSON.
"The condensations of 2 : 4-dimethylpyrrole." By J. R. SAXTON.
"Conjugation of a hetero-atom across a single bond." By C. A. COULSON and J. DE HEER.
"The cardiac glycosides of *Urechites suberecta*." By C. H. HASSALL.
"Dithiols. Part XI. The reactions of epoxides with hydroxy- and poly-thiols." By L. N. OWEN and P. N. SMITH.
"1 : 2-Diamino-4 : 5-dichlorobenzene." By W. DAVIS and W. C. J. ROSS.
"Sesquiterpenoids. Part I. Evidence for a nine-membered ring in caryophyllene." By D. H. R. BARTON and A. S. LINDSEY.
"The nature of the co-ordinate link. Part IV. Complex formation by phosphorus trifluoride." By J. CHATT and A. A. WILLIAMS.
"Iron di*cyclo*pentadienyl." By S. MILLER, J. TEBBOTH, and J. TREMAINE.
"Titanium tetrachloride as a catalyst in the Friedel–Crafts reaction. Part I. Acylation." By N. M. CULLINANE, S. J. CHARD, and D. M. LEYSHON.
"Non-catalytic reduction of thiophens. Part II. 2-Methylthiophen, 3-methylthiophen, and 2 : 5-dimethylthiophen." By S. F. BIRCH and D. T. MCALLAN.
"The caryophyllenes. Part IX. Homocaryophyllenic acid." By T. L. DAWSON and G. R. RAMAGE.
"The rate of dehydration of chrome alum." By M. M. T. ANOUS, R. S. BRADLEY, and (the late) J. COLVIN.
"The chlorination of β-D-glucopyranose penta-acetate. Abnormal reactions of phosphorus pentachloride." By R. A. ABRAMOVITCH.
"Aromatic hydrocarbons. Part LXII. 1 : 2-3 : 4-9 : 10-tribenzopyrene." By E. CLAR and J. R. HOLKER.
"Reactions of methazonic acid. Part II. Some 3-aminolepidines, 3-amino-4-arylquinolines, and derived compounds." By K. SCHOFIELD and R. S. THEOBALD.
"Triterpene resinols and related acids. Part XXIII. (*a*) The decarboxylation of acetylketo-ursolic acid. (*b*) The oxidation of methyl acetylursolate with hydrogen peroxide." By W. MANSON and F. S. SPRING.
"Triterpene resinols and related acids. Part XXIV. 18-*iso*-β-Amyranol." By R. BUDZIAREK, W. MANSON, and F. S. SPRING.
"Nucleophilic displacement of fluorine from organic compounds. Part I. Kinetics of the reactions of 1-fluoro-2 : 4-dinitrobenzene with primary aromatic amines." By N. B. CHAPMAN and R. E. PARKER.
"Simple analogues of cortisone. Part I. Some monocyclic compounds." By J. D. BILLIMORIA and N. F. MACLAGAN.
"Substituted benzidines and related compounds as reagents in analytical chemistry. Part VIII. 3 : 3'-Dimethylnaphthidine as indicator in the titration of cadmium, calcium, and indium with ferrocyanide." By R. BELCHER, A. J. NUTTEN, and W. I. STEPHEN.
"Infra-red studies of the molecular interactions of ω-hydroxypalmitic acid." By W. J. ORVILLE THOMAS.
"Electron distribution in conjugated free radicals." By JULIANE JACOBS.
"Polyene acids. Part II. The stereochemistry of the β-methylmuconic acids." By J. A. ELVIDGE, R. P. LINSTEAD, and P. SIMS.
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"Preparation and infra-red spectra of the geometrical isomers of 5-oxanon-3-ene (but-1-enyl butyl ether)." By R. H. HALL, A. R. PHILPOTTS, E. S. STERN, and W. THAIN.
"The kinetics of chlorohydrin formation. Part III. The reaction between hypochlorous acid and crotonic acid at constant pH." By D. A. CRAW and G. C. ISRAEL.

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"9 : 10-Dihydrophenanthrenes. Part II. 9 : 10-Dihydro-4 : 5-dimethoxyphenanthrene." By D. M. HALL and E. E. TURNER.
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"Cobaltic acetate. Part I." By J. A. SHARP and A. G. WHITE.
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chemistry. [Revised edition with supplement]. Johannesburg 1951. pp. 68. [Typescript]. (*Reference.*) From the Author.

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